PROTEASE VARIANTS

FIELD OF THE INVENTION

The present invention relates to variants of proteases belonging to the RP-II or C-component type, and methods for the construction of such variants with altered properties, such as stability (e.g. thermostability or storage stability), Ca^{2+} dependency, and pH dependent activity.

BACKGROUND OF THE INVENTION

Enzymes have been used within the detergent industry as part of washing formulations for more than 30 years. Proteases are from a commercial perspective the most relevant enzyme in such formulations, but other enzymes including lipases, amylases, cellulases, hemicellulases or mixtures of enzymes are also often used. Proteases are also used in other fields, such as production of diary products, processing of hides, feed processing, etc.

To improve the cost and/or the performance of proteases there is an ongoing search for proteases with altered properties, such as increased activity at low temperatures, increased thermostability, increased specific activity at a given pH, altered Ca^{2+} dependency, increased stability in the presence of other detergent ingredients (e.g. bleach, surfactants etc.), modified specificity in respect of substrates, etc.

The search for proteases with altered properties includes both discovery of naturally occurring proteases, i.e. so called wild-type proteases but also alteration of well-known proteases by e.g. genetic manipulation of the nucleic acid sequence encoding said proteases. Knowledge of the relationship between the three-dimensional structure and the function of a protein has improved the ability to evaluate which areas of a protein to alter to affect a specific property of the protein.

One group of proteases, which has been indicated for use in detergents, food processing, feed processing is the RP-II proteases or C-component proteases belonging to the protease family S1B, glutamic-acid-specific endopeptidases. This family has till now only received relatively minor attention and has not been further grouped into different sub-groups. However, from the amino acid identities of isolated RP-II proteases it is evident that subgroups exist. Bacillus proteases of the RP-II type are serine proteases that in primary structure are similar to chymotrypsin.

The first description of a protease of the RP-II family of Bacillus proteases was in US Patent No. 4,266,031 (Tang et al., Novo Industri A/S), where it was designated Component C and tentatively (and incorrectly) characterised as not being a serine protease or metallo protease. Component C was considered a contaminant in the production of the Bacillus licheniformis alkaline protease, subtilisin Carlsberg.

In EP 369 817 (Omnigene Bioproducts, Inc.) the *B. subtilis* member of the RP-II family was identified by its amino acid and DNA sequences. The enzyme was again stated not to be a serine protease, and the family name RP-II designated (Residual Protease II). The enzyme was characterized further as a metallo protease by the inventors of EP 369 817 (Rufo et al., 1990, J. Bacteriol. 2 1019-1023, and Sloma et al., 1990, J. Bacteriol. 172 1024-1029), designating the enzyme as mpr.

In WO 91/13553 (Novozymes A/S) the amino acid sequence of the C component was disclosed, stating that it is a serine protease specific for glutamic and aspartic acid, while EP 482 879 (Shionogi & Co. Ltd.) disclosed the enzyme and a DNA sequence encoding the C component from *B. licheniformis* ATCC No. 14580, naming the enzyme BLase. In EP 482 879 the protease is described as being specific for glutamic acid (see also Kakudo et al. "Purification, characterization, cloning, and expression of a glutamic acid-specific protease from Bacillus licheniformis ATCC 14580". J. Biol. Chem. 267:23782 (1992)).

In 1997 Okamoto et al. (Appl. Microbiol. Biotechnol. (1997) 48 27-33) found that the *B. subtilis* homologue of BLase, named BSase was identical to the above-mentioned enzyme, mpr/RP-II.

In 1999 Rebrikov et al. (Journal of Protein Chemistry, Vol. 18, No. 1, 1999) disclosed a Glu-specific protease from *B. intermedius* that also belongs to the RP-II family.

In WO 01/16285 a number of further RP-II protease were disclosed with DNA and amino acid sequences. These RP-II proteases were isolated from *B. pumilus*, *B. halmapalus* and *B. licheniformis*. WO 01/16285 also discloses a number of variants of RP-II proteases. These variants were based on various concepts relating to the primary structure of the RP-II proteases (amino acid sequences).

The homology matrix in Table 1 below clearly indicates that the RP-II proteases 1 to 8 are a distinct group of Glu-specific proteases that are clearly different from the other Glu-specific proteases in the Matrix

Table 1

	1	2	3	4	5	6	7	8	9	10	11	12	13
1	**100**	**99**	**97**	**60**	**55**	**55**	**47**	**59**	46	45	45	47	49
2		**100**	**99**	**60**	**60**	**59**	**50**	**61**	50	44	45	46	52
3			**100**	**60**	**57**	**54**	**47**	**60**	47	45	45	44	49
4				**100**	**94**	**92**	**68**	**57**	44	38	40	42	47
5					**100**	**91**	**59**	**54**	44	42	40	43	45
6						**100**	**63**	**53**	39	42	46	41	45
7							**100**	**48**	41	41	40	36	44
8								**100**	50	45	46	46	54
9									***100***	***63***	***53***	***55***	***49***
10										***100***	***53***	***56***	***52***
11											***100***	***78***	***54***
12												***100***	***53***
13													***100***

In the matrix the sequences are identified by the patent publication in which first published or sequence database accession numbers.

1. *Bacillus sp. JA96* glutamic-acid-specific endopeptidase, JA96, WO 01/16285
2. 1p3e *B. Intermedius*, glutamic-acid-specific endopeptidase, BIP, EMBL No. Y5136, Rebrikov et al., Journal of Protein Chemistry, Vol. 18, No. 1, 1999
3. *Bacillus sp.* BO32 glutamic-acid-specific endopeptidase, BO32, WO 01/16285
4. *Bacillus licheniformis*, BLC, WO 01/16285 (cf. US Patent No. 4,266,031)
5. *Bacillus sp.* CDJ31 glutamic-acid-specific endopeptidase, CDJ31, WO 01/16285
6. *Bacillus sp.* AC116 glutamic-acid-specific endopeptidase, AC116, WO 01/16285
7. mpr_bacsu *Bacillus subtilis* serine protease, MPR, EP 369 817
8. Bacillus sp. AA513 glutamic-acid-specific endopeptidase, AA513, WO 01/16285
9. eta_staau *Staphylococcus aureus* exfoliative toxin A (Lee et al. Sequence determination and comparison of the exfoliative toxin A and toxin B genes from *Staphylococcus aureus*; J. Bacteriol. 169:3904 (1987))
10. etb_staau *Staphylococcus aureus* exfoliative toxin B (Jackson,M.P.; Iandolo,J.J.; Sequence of the exfoliative toxin B gene of *Staphylococcus aureus*; J. Bacteriol.

167:726 (1986))

11. q53781 *Staphylococcus aureus* (strain Mu50 / ATCC 700699) (Rieneck et al.; Submitted (JUN-1996) to the EMBL/GenBank/DDBJ databases)

12. q53782 *Staphylococcus aureus* (strain Mu50 / ATCC 700699) (Rieneck et al.,"Molecular cloning and expression of a novel *Staphylococcus aureus* antigen". Biochim. Biophys. Acta 1350:128 (1997)

13. stsp_staau Staphylococcal serine endoproteinase V8 Glu-C (Gray, "Nucleotide sequence of the serine protease gene of *Staphylococcus aureus*, strain V8" Nucleic Acids Res. 15:6757 (1987)

The three-dimensional structure of the protease Toxin A from *Staphylococcus aureus*. Belonging to the S1B family has been determined by Cavarelli, J., et al. Structure Vol. 5, p. 813 1997.

However, despite the sequence homology between the proteases belonging to the RP-II proteases and Toxin A from *Staphylococcus aureus*, modelling of the three-dimensional structure of RP-II proteases on the basis of the three-dimensional structure of Toxin A from *Staphylococcus aureus* may result in an incorrect three-dimensional structure because of structural differences, especially because the distinct difference in sequence homology to the RP-II proteases.

The inventors of the present invention have elucidated the three-dimensional structure of the C-component protease from *Bacillus licheniformis* and found that there are several differences between this and the three-dimensional structure of Toxin A from *Staphylococcus aureus* also belonging to the S1B subgroup of proteases. This surprising difference in structure makes it advantageous to use the BLC structure as basis for homology modelling of RP-II proteases, which, in turn, will improve the ability to obtain desired changes in functionality by protein engineering.

BRIEF DESCRIPTION OF THE INVENTION

The inventors have modified the amino acid sequence of a RP-II protease to obtain variants with improved properties, based on the three-dimensional structure of the C-component. The variants will have altered properties, such as increased activity at low temperatures, increased thermostability, increased specific activity at a given pH, altered Ca^{2+} dependency, increased stability in the presence of other detergent ingredients (e.g. bleach, surfactants etc.) etc.

Accordingly, the object of the present invention is to provide a method for constructing RP-II proteases having altered properties, in particular to provide a method for constructing RP-II proteases having altered properties as described above.

Thus, in its broadest aspect, the present invention relates to a method for constructing a variant of a parent RP-II protease, wherein the variant has at least one altered property as compared to said parent RP-II protease, which method comprises:

i) analyzing the three-dimensional structure of the RP-II protease to identify, on the basis of an evaluation of structural considerations, at least one amino acid residue or at least one structural region of the RP-II protease, which is of relevance for altering said property;

ii) constructing a variant of the RP-II protease, which as compared to the parent RP-II protease, has been modified in the amino acid residue or structural part identified in i) so as to alter said property; and

iii) testing the resulting RP-II protease variant for said property.

Although it has been described in the following that modification of the parent RP-II protease in certain regions and/or positions is expected to confer a particular effect to the thus produced RP-II protease variant, it should be noted that modification of the parent RP-II protease in any of such regions may also give rise to any other of the above-mentioned effects. For example, any of the regions and/or positions mentioned as being of particular interest with respect to, e.g., improved thermostability, may also give rise to, e.g., higher activity at a lower pH, an altered pH optimum, or increased specific activity, such as increased peptidase activity.

Further aspects of the present invention relates to variants of a RP-II protease, the DNA encoding such variants and methods of preparing the variants. Still further aspects of the present invention relates to the use of the variants for various industrial purposes, in particular as an additive in detergent compositions. Other aspects of the present invention will be apparent from the below description as well as from the appended claims.

BRIEF DESCRIPTION OF DRAWINGS

Fig. 1 provides a schematic structure of the RP-II protease from Bacillus licheniformis, BLC.

Fig. 2 shows a 3D structure based alignment of the wild type RP-II proteases 1 to 8 of Table 1.

Fig. 3 shows the BLC protease ribbon structure in black, with indication of active site residues, the bound peptide and the ion-binding site. The calcium ion is the sphere at the bottom of the Figure, the active site residues are in light grey and shown in stick model, and the bound peptide DAFE is in medium grey and shown in stick model.

BRIEF DESCRIPTION OF APPENDICES

APPENDIX 1 provides the structural coordinates for the solved crystal 3D structure of the BLC RP-II protease, in the standard pdb format. The residues are numbered from 1-217, the calcium ion is numbered 301, and the DAFE substrate is numbered 401-404.

DEFINITIONS

Prior to discussing this invention in further detail, the following terms and conventions will first be defined.

For a detailed description of the nomenclature of amino acids and nucleic acids and modifications introduced in a polypeptide or protein and especially in a RP-II protease by genetic manipulation, we refer to WO 01/16285 pages 5 to 15, hereby incorporated by reference.

The term "RP-II proteases" refers to a sub-group of serine protease, belonging to the protease family S1B, glutamic-acid-specific endopeptidases. Serine proteases or serine peptidases is a subgroup of proteases characterised by having a serine in the active site, which forms a covalent adduct with the substrate. Further the RP-II proteases (and the serine proteases) are characterised by having two active site amino acid residues apart from the serine, namely a histidine and an aspartic acid residue.

The RP-II proteases have a homology to the rest of the S1B protease family of around 50% (using the UWGCG version 8 software GAP program), or more preferred a homology higher than 55%. Table 1 demonstrate homologies between various S1B proteases. The RP-II proteases, nos. 1 to 8, are in Table 1 indicated in bold and the other S1B proteases, nos. 9 to 13, in bold italics. Table 1 shows that there is a clear distinction to the RP-II proteases from the other S1B proteases, but it is also clear that among the RP-II proteases there are subgroups. One subgroup comprises nos. 1, 2, and 3; and another subgroup comprises nos. 4, 5, and 6. The lengths of the listed RP-II proteases vary from 215 to 222 amino acid residues and experience within the subtilisin subgroups of subtilases indicates that such a variation in length probably has only

little effect on the 3-dimensional structures of these and other RP-II protease sub-groups.

PARENT

The term "parent" is in the context of the present invention to be understood as a protein, which is modified to create a protein variant. The parent protein may be a naturally occurring (wild-type) polypeptide or it may be a variant thereof prepared by any suitable means. For instance, the parent protein may be a variant of a naturally occurring protein which has been modified by substitution, chemical modification, deletion or truncation of one or more amino acid residues, or by addition or insertion of one or more amino acid residues to the amino acid sequence, of a naturally-occurring polypeptide. Thus the term "parent RP-II protease" refers to a RP-II protease which is modified to create a RP-II protease variant.

VARIANT

The term "variant" is in the context of the present invention to be understood as a protein which has been modified as compared to a parent protein at one or more amino acid residues.

MODIFICATION

The term "modification(s)" or "modified" is in the context of the present invention to be understood as to include chemical modification of a protein as well as genetic manipulation of the DNA encoding a protein. The modification(s) may be replacement(s) of the amino acid side chain(s), substitution(s), deletion(s) and/or insertions in or at the amino acid(s) of interest. Thus the term "modified protein", e.g. "modified RP-II protease", is to be understood as a protein which contains modification(s) compared to a parent protein, e.g. RP-II protease.

HOMOLOGY

"Homology" or "homologous to" is in the context of the present invention to be understood in its conventional meaning and the "homology" between two amino acid sequences should be determined by use of the "Similarity" parameter defined by the GAP program from the University of Wisconsin Genetics Computer Group (UWGCG)

package using default settings for alignment parameters, comparison matrix, gap and gap extension penalties. Default values for GAP penalties, i.e. GAP creation penalty of 3.0 and GAP extension penalty of 0.1 (Program Manual for the Wisconsin Package, Version 8, August 1994, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711). The method is also described in S.B. Needleman and C.D. Wunsch, Journal of Molecular Biology, 48, 443-445 (1970). Identities can be extracted from the same calculation. The homology between two amino acid sequences can also be determined by "identity" or "similarity" using the GAP routine of the UWGCG package version 9.1 with default setting for alignment parameters, comparison matrix, gap and gap extension penalties can also be applied using the following parameters: gap creation penalty = 8 and gap extension penalty = 8 and all other parameters kept at their default values. The output from the routine is besides the amino acid alignment the calculation of the "Percent Identity" and the "Similarity" between the two sequences. The numbers calculated using UWGCG package version 9.1 is slightly different from the version 8.

NAMING OF RP-II PROTEASES

In describing the RP-II proteases of the invention the following abbreviations are used for ease of reference:

BLC = RP-II protease from *Bacillus licheniformis* (US Patent No. 4,266,031),
AA513 = RP-II protease from *Bacillus halmapalus* AA513 (WO 01/16285),
AC116 = RP-II protease from *Bacillus licheniformis* AC116 (WO 01/16285)
BO32 = RP-II protease from *Bacillus pumilus* BO32 (WO 01/16285),
CDJ31 = RP-II protease from *Bacillus licheniformis* CDJ31 (WO 01/16285),
JA96 = RP-II protease from *Bacillus pumilus* JA96 (WO 01/16285),
MPR = RP-II protease from *Bacillus subtilis* IS75 (EP 369 817 B1)
BIP = RP-II protease from *B. intermedius* (Rebrikov et al., Journal of Protein Chemistry, Vol. 18, No. 1, 1999)

SEQUENCE LISTING

In the appended Sequence Listing the RP-II proteases are indicated as:
SEQ. ID. NO. 1 = BLC (DNA), SEQ. ID. NO. 2 = BLC (AA),
SEQ. ID. NO. 3 = AA513 (DNA), SEQ. ID. NO. 4 = AA513 (AA),
SEQ. ID. NO. 5 = AC116 (DNA), SEQ. ID. NO. 6 = AC116 (AA)

SEQ. ID. NO. 7 = BO32 (DNA), SEQ. ID. NO. 8 = BO32 (AA)

SEQ. ID. NO. 9 = CDJ31 (DNA), SEQ. ID. NO. 10 = CDJ31 (AA)

SEQ. ID. NO. 11 = JA96 (DNA), SEQ. ID. NO. 12 = JA96 (AA)

SEQ. ID. NO. 13 = BSMPR (DNA), SEQ. ID. NO. 14 = BSMPR (AA)

SEQ. ID. NO. 15 = BIP (DNA), SEQ. ID. NO. 16 = BIP (AA)

POSITION

The term "position" is in the context of the present invention to be understood as the number of an amino acid residue in a peptide, polypeptide or protein when counting from the N-terminal end of said peptide/polypeptide. The position numbers used here normally refer directly to different RP-II proteases.

The RP-II proteases are numbered individually according to each of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, and 16.

Corresponding position

The invention, however, is not limited to variants of these particular RP-II proteases but extends to parent proteases containing amino acid residues at positions which are "equivalent" to the particular identified residues in *Bacillus licheniformis* RP-II protease. In some preferred embodiment of the present invention, the parent protease is JA96 or BIP RP-II protease and the substitutions are made at the equivalent amino acid residue positions in JA96 or BIP corresponding to those listed above.

A residue (amino acid) position of a RP-II protease is equivalent to a residue (position) of the *Bacillus licheniformis* RP-II protease if it is either homologous (i.e., corresponding in position in either primary or tertiary structure) or analogous to a specific residue or portion of that residue in *Bacillus licheniformis* RP-II protease (i.e., having the same or similar functional capacity to combine, react, or interact chemically).

In order to establish homology to primary structure, the amino acid sequence of a precursor protease is directly compared to the *Bacillus licheniformis* RP-II protease, BLC, primary sequence by aligning the amino acid sequence of an isolated or parent wild type enzyme with a suitable well-known enzyme of the same group or class of enzymes defines a frame of reference. This type of numbering was used in WO 01/16285. If nothing else is indicated herein, in the present instance the *Bacillus licheniformis* RP-II protease, first designated component C and therefore here abbreviated BLC, has been chosen as standard.

In order to establish homology to the tertiary structure (3D structure) of BLC, the 3D structure based alignment in Fig. 2 has been provided. By using this alignment the amino acid sequence of a precursor RP-II protease may be directly correlated to the *Bacillus licheniformis* RP-II protease, BLC, primary sequence. For a novel RP-II protease sequence, the (3D based) position corresponding to a position in BLC is found by

i) identifying the RP-II protease from the alignment of Fig. 2 that is most homologous to the novel sequence,

ii) aligning the novel sequence with the sequence identified to find the corresponding position in the RP-II protease from Fig. 2, and

iii) establishing from Fig. 2 the corresponding position in BLC.

For comparison and finding the most homologous sequence the GAP program from GCG package as described below are used.

The alignment can as indicated above be obtained by the GAP routine of the GCG package version 8 to number the variants using the following parameters: gap creation penalty = 3 and gap extension penalty = 0.1 and all other parameters kept at their default values.

The alignment of Fig. 2 defines a number of deletions and insertions in relation to the sequence of BLC. In the alignment deletions are indicated by asterixes (*) in the referenced sequence, and the referenced enzyme will be considered to have a gap at the position in question. Insertions are indicated by asterixes (*) in the BLC sequence, and the positions in the referenced enzyme are given as the position number of the last amino acid residue where a corresponding amino acid residue exists in the standard enzyme with a lower case letter appended in alphabetical order, e.g. 82a, 82b, 82c, 82d, see Fig. 2.

In case the referenced enzyme contains a N- or C-terminal extension in comparison to BLC; an N-terminal extension is given the position number 0a, 0b, etc. in the direction of the N-terminal; and a C-terminal extension will be given either the position number of the C-terminal amino acid residue of BLC with a lower case letter appended in alphabetical order, or simply a continued consecutive numbering.

Thus for comparisons RP-II proteases are numbered by reference to the positions of the BLC RP-II protease (SEQ ID NO: 2) as provided in Fig. 2. The position is then indicated as "corresponding to BLC".

DETAILED DESCRIPTION OF THE INVENTION

The inventors of the present invention have elucidated the three-dimensional structure of BLC, SEQ ID NO:2 by X-ray crystallography and found that there are several interesting features in the structure of this protease in comparison with the known structures of other proteases, such as the RP-II proteases. These features include both similarities and differences.

RP-II proteases

As described above a RP-II protease is in the context of the present invention to be understood as a protease which has at least 50% homology to BLC (SEQ ID NO:2). In particular said protease may have at least 55% homology to BLC, i.e. to SEQ ID NO:2. The invention thus relates to variant RP-II proteases having at least 50% homology to BLC.

Specifically the variants of the invention may comprise RP-II proteases comprising a number of modifications or modifications in a number of positions ranging from at least one and up to 50, or from 1 to 45, or from 1 to 40, or from 1 to 35, or from 1 to 30, or from 1 to 25, or from 1 to 20, or from 1 to 15, or from 1 to 14, or from 1 to 13, or from 1 to 12, or from 1 to 11, or from 1 to 10, or from 1 to 9, or from 1 to 8, or from 1 to 7, or from 1 to 6, or from 1 to 5, or from 1 to 4, or from 1 to 3, or from 1 to 2 modifications or positions. Such modifications comprising substitutions, deletions and insertions in the indicated number or number of positions.

A RP-II protease variant of the present invention is encoded by an isolated polynucleotide, which nucleic acid sequence has at least 50% homology with the nucleic acid sequence shown in SEQ ID NO: 1, and where the polynucleotide encodes a variant RP-II protease in relation to a parent protease.

In a first embodiment of the present invention a RP-II protease suitable for the purpose described herein may be a RP-II protease homologous to the three-dimensional structure of BLC, i.e. it may be homologous to the three-dimensional structure defined by the structure coordinates in Appendix 1 by comprising the structural elements defined below.

It is well-known to a person skilled in the art that a set of structure coordinates for a protein or a portion thereof is a relative set of points that define a shape in three dimensions; it is possible that an entirely different set of coordinates defines an identical or a similar shape. Moreover, slight variations in the individual coordinates may have little or no effect on the overall shape.

These variations in coordinates may be generated because of mathematical manipulations of the structure coordinates. For example, the structure coordinates of Appendix 1 (BLC structure) may be manipulated by crystallographic permutations of the structure coordinates, fractionalization of the structure coordinates, integer additions or subtractions to sets of the structure coordinates, inversion of the structure coordinates or any combination of the above. Alternatively, said variations may be due to differences in the primary amino acid sequence.

When such variations are within an acceptable standard error as compared to the structure coordinates of Appendix 1 said three-dimensional structure is within the context of the present invention to be understood as being homologous to the structure of Appendix 1. The standard error may typically be measured as the root mean square deviation of e.g. conserved backbone residues, where the term "root mean square deviation" (RMS) means the square root of the arithmetic mean of the squares of the deviations from the mean.

It is also well-known to a person skilled in the art that within a group of proteins which have a homologous structure there may be variations in the three-dimensional structure in certain areas or domains of the structure, e.g. loops, which are not, or at least only of a small importance to the functional domains of the structure, but which may result in a big root mean square deviation of the conserved residue backbone atoms between said structures.

Thus it is well known that a set of structure coordinates is unique to the crystallised protein. No other three dimensional structure will have the exact same set of coordinates, be it a homologous structure or even the same protein crystallised in different manner. There are natural fluctuations in the coordinates. The overall structure and the inter-atomic relationship can be found to be similar. The similarity can be discussed in terms of root mean square deviation of each atom of a structure from each "homologous" atom of another structure. However, only identical proteins have the exact same number of atoms. Therefore, proteins having a similarity below 100% will often have a different number of atoms, and thus the root mean square deviation can not be calculated on all atoms, but only the ones that are considered "homologous". A precise description of the similarity based on the coordinates is thus difficult to describe and difficult to compute for homologous proteins. Regarding the present invention, similarities in 3D structure of different RP-II proteases can be described by the content of homologous structural elements, and/or the similarity in amino acid or DNA sequence

Examples of BLC like RP-II proteases include the BLC = RP-II protease from

Bacillus licheniformis (cf. US Patent No. 4,266,031), AA513 = RP-II protease from *Bacillus halmapalus* AA513 (NP000368), AC116 = RP-II protease from *Bacillus licheniformis* AC116 (NP000364), BO32 = RP-II protease from *Bacillus pumilus* BO32 (NP000366), CDJ31 = RP-II protease from *Bacillus licheniformis* CDJ31 (NP000365), JA96 = RP-II protease from *Bacillus pumilus* JA96 (NP000367), MPR = RP-II protease from *Bacillus subtilis* IS75 (cf. EP 369 817 B1), BIP = RP-II protease from *B. intermedius* (EMBL No. Y5136, Rebrikov et al., Journal of Protein Chemistry, Vol. 18, No. 1, 1999)

Accordingly, a preferred embodiment of the present invention is a variant of a parent RP-II protease or a RP-II protease variant which is at least 50% homologous to the sequence of SEQ ID NO 2 preferably at least 55%, preferably at least 65%, at least 70%, at least 74%, at least 80%, at least 83%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% homologous to the sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14 or 16.

A further embodiment of the invention is a RP-II protease variant comprising the following structural characteristics:

a) two beta-barrel domains each comprising six long strands in antiparallel organisation,
b) three alpha helices,
c) at least one ion-binding site,
d) an active site comprising the amino acid residues His, Asp and Ser.

The potential ion binding site is defined as similar coordination or arrangement of the coordinates as in the 3D structure of BLC having one calcium ion coordinated by the Ile 3 carbonyl atom O, the Ser 5 carbonyl atom O and bidendate by the Asp 161 Carboxyl acid group and the further coordination made by waters. The calcium may be substituted in the structure by water but then having the same coordination.

The RP-II protease variants of the present invention are encoded by isolated polynucleotides, which nucleic acid sequence has at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% homology with the nucleic acid sequence shown in SEQ ID NO:1, 3, 5, 7 ,9, 11, 13, or 15, and where the polynucleotide encodes a variant RP-II protease in relation to a parent protease.

Further the isolated nucleic acid sequence encoding a RP-II protease variant of

the invention hybridizes with a complementary strand of the nucleic acid sequence shown in SEQ ID NO: 1 preferably under low stringency conditions, at least under medium stringency conditions, at least under medium/high stringency conditions, at least under high stringency conditions, at least under very high stringency conditions.

Suitable experimental conditions for determining hybridization at low, medium, or high stringency between a nucleotide probe and a homologous DNA or RNA sequence involves presoaking of the filter containing the DNA fragments or RNA to hybridize in 5 x SSC (Sodium chloride/Sodium citrate, Sambrook et al. 1989) for 10 min, and prehybridization of the filter in a solution of 5 x SSC, 5 x Denhardt's solution (Sambrook et al. 1989), 0.5 % SDS and 100 µg/ml of denatured sonicated salmon sperm DNA (Sambrook et al. 1989), followed by hybridization in the same solution containing a concentration of 10ng/ml of a random-primed (Feinberg, A. P. and Vogelstein, B. (1983) *Anal. Biochem.* 132:6-13), ^{32}P-dCTP-labeled (specific activity > 1 x 10^9 cpm/µg) probe for 12 hours at ca. 45°C. The filter is then washed twice for 30 minutes in 2 x SSC, 0.5 % SDS at least 55°C (low stringency), more preferably at least 60°C (medium stringency), still more preferably at least 65°C (medium/high stringency), even more preferably at least 70°C (high stringency), and even more preferably at least 75°C (very high stringency).

Three-dimensional structure of RP-II proteases

The BLC RP-II protease was used to elucidate the three-dimensional structure forming the basis for the present invention.

The structure of BLC was solved in accordance with the principle for x-ray crystallographic methods, for example, as given in X-Ray Structure Determination, Stout, G.K. and Jensen, L.H., John Wiley & Sons, Inc. NY, 1989.

The structural coordinates for the solved crystal structure of BLC are given in standard PDB format (Protein Data Bank, Brookhaven National Laboratory, Brookhaven, CT) as set forth in Appendix 1. It is to be understood that Appendix 1 forms part of the present application. In the context of Appendix 1, the following abbreviations are used: CA refers to c-alpha (carbon atoms) or to calcium ions, (however to avoid misunderstandings we normally use the full names "c-alpha atoms", "calcium" “Ca” or "ion" in the present specification). Amino acid residues are given in their standard three-letter code or the standard one-letter code. The structural coordinates in Appendix 1 contain the protease structure wherein the active serine was replaced by alanine and a com-

plex formed with the peptide DAFE (= Asp-Ala-Phe-Glu) as well as water molecules. The protease coordinates has a chain identification called A, whereas the peptide is called B, the calcium ion is called C, and the water is W. In the following the positions of the mentioned residues refer to the sequence of BLC as disclosed in SEQ ID NO: 2.

The overall structure of BLC falls into the S1 group of the proteases (MEROPS; http://merops.sanger.ac.uk/). The structure is a trypsin type of fold with two beta-barrel domains. The beta-barrel's each consists of six antiparallel beta-sheets folded into a beta-barrel. The topology can be described as S1-S2-S3-S6-S5-S4 for the strands in both beta-barrels. It is assumed that all the RP-II proteases fall within the same general overall structure.

The 3D structure of C-component serine protease from *Bacillus licheniformis* has 16 strands of which the 12 bigger strands compose the two beta-barrels; and 3 helixes. The four very short strands are number 1, 5, 6 and 10 counting from the N-terminal and are composed of residue numbers 9-10, 50-51, 56-57 and 114-115. The other strands are residue numbers 22-26, 31-36, 41-44, 62-65, 77-83, 99-102, 126-131, 142-151, 156-159, 171-177, 182-192 and 201-205. One main helix C-terminal residue number 208-219. Two very small helices are composed of residues 86-90 and 106-110.

The active site consists of a triad involving the Ser in position 167, the His in position 47, and the Asp in position 96.

The 3D structure of BLC has one calcium ion coordinated by the carbonyl oxygen atom of Ile in position 3, the carbonyl oxygen atom of Ser in position 5, and bidendate by the Carboxylic acid group of Asp in position 161. Further coordinations are made by water molecules.

The calcium ion is placed in a distance from the CA atoms of the active site and Gly in position 168 as provided below:

Ser 167 CA atom to Ca ion: 16.07Å

His 47 CA atom to Ca ion: 24.27Å

Asp 96 CA atom to Ca ion: 23.72Å

Gly 168 CA atom to Ca ion: 19.20Å

The position of an ion-binding site can be defined by the distance to four specific atoms in the core structure. The distance from the ion-binding site to the c-alpha atoms of the three active site residues has been chosen. Throughout the RP-II proteases the residues Ser, His and Asp in the active site are highly conserved. In BLC they are Asp96, His47 and Ser167. The fourth distance chosen is the distance to the c-alpha

atom of the amino acid residue coming first after the active site serine residue in the sequence (herein after called "next to Ser"); in the 3D structure of BLC it is Gly168.

In a preferred embodiment of the present invention, the distance between the ion-binding site and i) Asp c-alpha atom is 22.50-24.00 Å, ii) His c-alpha atom is 23.25-25.25 Å, iii) Ser c-alpha atom is 15.00-17.00Å, iv) next to Ser c-alpha atom is 18.20-20.20 Å,

However these distances may vary from one RP-II protease to the other, and as described above, the ion binding site may also bind to a sodium ion. The present distances are given with a calcium ion in the structure. If a sodium ion was bound instead the distances would be shifted a little bit. Generally the distances can vary ±0.8Å, preferably ±0.7Å, ±0.6Å, ±0.5Å, ±0.4Å, or most preferably ±0.3Å.

Further, in the RP-II proteases, the peptide structure circumscribing the ion-binding site is composed of the amino acid residues placed in positions 1-7, 159-162 and 143-145 with the coordinating atoms being the backbone carbonyl oxygen atom of residues I3, S5, D161 and water molecules.

3D structures of RP-II proteases can be modelled using the known structure of a related protease and general modelling tools as shown in Example 1. A prerequisite for obtaining a realistic 3D model structure is that the model is based on an adequate sequence homology higher than 50%, preferably higher than 55%, and even more preferred higher than 60% to the sequence of the protease for which the structure is known. RP-II Protease models can be constructed based on the 3D guided sequence alignments to BLC in Figure 2.

Therefore 3D structure models of RP-II proteases could in principle be made by using the modelling tools and the known 3D structure of the toxin A protease from *Staphylococcus aureus* from the Exf family of proteases (Cavarelli et al. (1997) The Structure of *Staphylococcus aureus* Epidermolytic Toxin A, an atypic serine protease, at 1.7 Å resolution, Structure, Vol. 5, p.813 (pdb name 1ARP).

If compared to the structure of the toxin A protease from Staphylococcus aureus, the structure of the RP-II proteases, as represented by BLC, can be divided into a "common protease" region, an "intermediate" region and a "nonhomologous" region.

The active site can be found in the common protease region, which is structurally closely related to the Toxin A structure. The common protease region is composed of residues 58, 70-83. The common protease region has an RMS lower than 1.2.

Outside the common protease region the structure of the RP-II protease BLC differs from the Toxin A structure to a greater extent.

The intermediate region consists of residues 14-28, 29-51, 94-104, 155-175. The intermediate region has an RMS bigger than 1.2 and less than 1.8. Any relationships between the three-dimensional structure and functionality based on modelling from the S. aureus 3D structure are potentially difficult to predict in this region of the RP-II proteases.

The common region and the intermediate region consist of the majority of the two central beta-barrels, especially the strands of the beta-barrels.

The nonhomologous region consists of residues 1-6, 7-13, 52-57, 59-69, 84-88, 89-93, 105-153. The nonhomologous region has a RMS higher than 1.5. Any relationships between the three-dimensional structure and functionality based on modelling from the S. aureus 3D structure are very difficult to predict in this region of the RP-II proteases.

Inferred structure-function relationships based on model building of a RP-II protease 3D structure on the 3D structure of S. aureus Toxin A would thus be very uncertain and speculative.

Homology building of RP-II proteases

A model structure of a RP-II protease can be built using the BLC structure in Appendix 1, or a structure similar to the BLC structure comprising the structural elements (a) two beta-barrel domains each comprising six long strands in antiparallel organisation, (b) three alpha helices, (c) at least one low affinity ion-binding site, and (d) an active site comprising the amino acid residues His, Asp and Ser, or other 3D RP-II protease structures, e.g. established by X-ray structure determination, that may become available in the future, and the Homology™ program or a comparable program, e.g., Modeller™ (both from Molecular Simulations, Inc., San Diego, CA). The principle is to align the amino acid sequence of a protein for which the 3D structure is known with the amino acid sequence of a protein for which a model 3D structure has to be constructed. The structurally conserved regions can then be built on the basis of consensus sequences. In areas lacking homology, loop structures can be inserted, or sequences can be deleted with subsequent bonding of the necessary residues using, e.g., the program Homology. Subsequent relaxation and optimization of the structure should be done using either Homology or another molecular simulation program, e.g., CHARMm™ from Molecular Simulations.

Methods for designing BLC and RP-II or S1B family protease variants

Comparisons of the molecular dynamics of different proteins can give a hint as to which domains are important or connected to certain properties pertained by each protein.

The present invention comprises a method of producing a variant of a parent BLC like RP-II protease, the variant having at least one altered property as compared to the parent BLC like RP-II protease, the method comprising:

a) producing a model structure of the parent BLC like RP-II protease on the three-dimensional structure of BLC,
b) comparing the model three-dimensional structure of the parent BLC like RP-II protease to the BLC structure by superimposing the structures through matching the active residues CA, CB, C, O, and N atoms,
c) identifying on the basis of the comparison in step a) at least one structural part of the parent BLC like RP-II protease, wherein an alteration in said structural part is predicted to result in an altered property;
d) modifying the nucleic acid sequence encoding the parent BLC like RP-II protease to produce a nucleic acid sequence encoding deletion or substitution of one or more amino acids at a position corresponding to said structural part, or an insertion of one or more amino acid residues in positions corresponding to said structural part;
e) expressing the modified nucleic acid sequence in a host cell to produce the variant RP-II protease;
f) isolating the produced protease;
g) purifying the isolated protease and
h) recovering the purified RP-II protease.

Stability - alteration of ion-binding site

An ion-binding site is a significant feature of an enzyme. Therefore alterations of the amino acid residues close to the ion-binding site are likely to result in alterations of the stability of the enzyme. Especially modifications affecting the charge distribution and/or the electrostatic field strength at or in the vicinity of the site are important.

Improved stability

Stabilisation of the ion-binding site of RP-II proteases may be obtained by modifications in positions close to the ion binding site.

Such modifications may comprise the substitution of a positively charged amino acid residue with a neutral or negatively charged residue, or the substitution of a neutral residue with a negatively charged residue or the deletion of a positively charged or neutral residue in positions close to the ion binding site.

Positions located at a distance of 10Å or less to the ion-binding site of BLC are: 1, 2, 3, 4, 5, 6, 7, 8, 143, 144, 145, 146, 158, 159, 160, 161, 162, 194, 199, 200, and 201. Especially positions 2, 3, 4, 5, 6, 7, 144, 159, 160, 161 located at a distance of 6 Å or less from the ion binding site are important.

Corresponding positions in other RP-II proteases may be identified using Fig. 2 herein.

The modifications D7E and D7Q in BLC are examples of suitable modifications in one of these positions.

Removal of ion-binding site in BLC

By removing the ion-binding site it is possible to alter the dependency of the enzyme on calcium or other ions in the solution.

Removal of the Calcium site in BLC can be done by the substitutions H144R and/or D161R,K+H144Q,N (SEQ ID NO: 2). Similar modifications may be made in structurally corresponding residues in other RP-II proteases.

Alteration of thermostability

A variant with improved stability (typically increased thermostability) may be obtained by modification of the mobility of identified regions, such as by introduction of disulfide bond(s), substitution with proline, alteration of hydrogen bond contact(s), altering charge distribution, introduction of salt bridge(s), filling in internal structural cavities with one or more amino acids with bulkier side groups (in e.g. regions which are structurally mobile), substitution of histidine residues with other amino acids, removal of a deamidation sites, or by helix capping.

Regions with increased mobility:

The below indicated regions of BLC have an increased mobility in the crystal

structure of the enzyme, and it is presently believed that these regions can be responsible for stability or activity of BLC and the other RP-II proteases. Especially thermostabilisation may be obtained by altering the highly mobile regions. Generally, thermostability may be improved by making these regions less mobile. Improvements of the enzyme may be obtained by making modifications in the regions and positions identified below. Introducing e.g. larger residues or residues having more atoms in the side chain could increase the stability, or, e.g., introduction of residues having fewer atoms in the side chain could be important for the mobility and thus the activity profile of the enzyme. The regions can be found by analysing the B-factors taken from the coordinate file in Appendix 1, and/or from molecular dynamics calculations of the isotropic fluctuations. These can be obtained by using the program CHARMm from MSI (Molecular Simulations Inc.).

Molecular dynamics simulation at 300K and 400K of BLC reveals the following highly mobile regions:

26-31, 50-55, 89-91, and 193-198, and 4-5, 11-12, 26-31, 50-55, 69-70, 89-91, 178-183, 195-199 and 216-221, respectively.

It is contemplated that modifications in these regions may influence the thermostability of RP-II proteases. Modifications are preferably made in the regions 26-31 (26, 27, 28, 29, 30, 31); 89-91 (89, 90, 91); 216-221 (216, 217, 218, 219, 220, 221), and especially in BLC the substitutions G30A and G91A. Similar modifications may be made in structurally corresponding residues in other RP-II proteases.

Also B-factors (see "in X-Ray Structure Determination, Stout, G.K. and Jensen, L.H., John Wiley & Sons, Inc. NY, 1989") from crystallographic data indicate the following more mobile regions in the BLC (RP-II protease) structure:

51-56, (i.e. 51, 52, 53, 54, 55, 56)
88-94, (i.e. 88, 89, 90, 91, 92, 93, 94)
118-122 (I. e. 118, 119, 120, 121, 122)
173-183 (i.e. 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183)

It is contemplated that modifications in these regions may influence the thermostability of RP-II proteases. Modifications are preferably made in the regions 51-56 and 118-122.

Disulfide bonds:

A RP-II protease variant of the present invention with improved stability, e.g.

thermostability, as compared to the parent RP-II protease may be obtained by introducing new inter-domain or intra-domain bonds to provide a more rigid and stable structure, such as by establishing inter- or intra-domain disulfide bridges. This is done by introducing cysteines in appropriate positions in the RP-II molecule by substitution(s) or insertion(s).

According to the guidelines mentioned above the below mentioned amino acid residues identified in the amino acid sequence of SEQ ID NO: 2 are contemplated as being suitable for cysteine replacement. With one or more of these substitutions with cysteine, disulfide bridges may form in a variant of BLC. A stabilising disulfide bridge may be constructed through the substitutions: S145C and T128C

Surface charge distribution

A variant with improved stability (typically improved thermostability or storage stability) as compared to the parent RP-II protease may be obtained by changing the surface charge distribution of the RP-II protease. For example, when the pH is lowered to about 5 or below, histidine residues typically become positively charged and, consequently, unfavorable electrostatic interactions on the protein surface may occur. By engineering the surface charge of the RP-II protease one may avoid such unfavorable electrostatic interactions that in turn may lead to a higher stability of the RP-II protease.

Charged amino acid residues are (a) positively charged: Lys, Arg, His (pH<5), Tyr (pH>9) and Cys (pH>10) and (b) negatively charged: Asp and Glu.

The surface charge distribution may be modified by (a) removing charged residues from the surface through deletion of a charged residue or substituting an uncharged residue for a charged residue, (b) adding charged residues to the surface through insertion of a charged residue or substituting a charged residue for an uncharged residue, or (c) by reverting the charge at a residue through substituting a positively charged residue for a negatively charged residue or substituting a negatively charged residue for a positively charged residue.

Therefore, a further aspect of the present invention relates to a method for constructing a variant of a parent RP-II protease having a modified surface charge distribution, the method comprising:

a) identifying, on the surface of the parent RP-II protease, at least one charged amino acid residue;

b) modifying the charged residue identified in step (a) through deletion or substitu-

tion with an uncharged amino acid residue;

c) optionally repeating steps a) and b) recursively;

d) preparing the variant resulting from steps a) - c);

e) testing the stability of said variant; and

f) optionally repeating steps a) - e) recursively; and

g) selecting a RP-II protease variant having increased stability as compared to the parent RP-II protease.

As will be understood by the skilled person it may also, in some cases, be advantageous to substitute an uncharged amino acid residue with an amino acid residue bearing a charge or, alternatively, it may in some cases be advantageous to substitute an amino acid residue bearing a charge with an amino acid residue bearing a charge of opposite sign. Thus, the above-mentioned method may be employed by the skilled person also for these purposes. In the case of substituting an uncharged amino acid residue with an amino acid residue bearing a charge the above-mentioned method may be employed the only difference being steps a) and b) which will then read:

a) identifying, on the surface of the parent RP-II protease, at least one position being occupied by an uncharged amino acid residue;

b) modifying the charge in that position by substituting the uncharged amino acid residue with a charged amino acid residue or by insertion of a charged amino acid residue at the position.

Also in the case of changing the sign of an amino acid residue present on the surface of the RP-II protease the above method may be employed. Again, compared to the above method, the only difference being steps a) and b) which, in this case, read:

a) identifying, on the surface of the parent RP-II protease, at least one charged amino acid residue;

b) substituting the charged amino acid residue identified in step (a) with an amino acid residue having an opposite charge.

In order to determine the amino acid residues of a protease, which are present on the surface of the enzyme, the surface accessible area are measured using the DSSP program (Kabsch and Sander, *Biopolymers* (1983), 22, 2577-2637). All residues having a surface accessibility higher than 0, 0.10, 0.20, 0.30, 0.35, 0.40, 0.45, 0.50, 0.55 or 0.60 are regarded a surface residue.

An amino acid residue found on the surface of BLC using the above method is T109 and it is contemplated that the substitutions T109R, K, H are of particular interest.

Similar substitutions may be introduced in equivalent positions of other RP-II proteases.

For the purpose of providing RP-II protease variants exhibiting improved wash performance it is possible to modify the pI of the RP-II protease through modification of the surface charge as indicated in WO 91/00345 (Novozymes A/S) and/or WO 99/20771 (Genencor International, Inc.)

Especially changing the pI of the RP-II protease is of interest

Changes in BLC:

T109R, K, H

Q143R, K, H

E209Q, N

D7N, S, T

Q174R, K, H

N216R, K, H

Y17R, K, H

Y95R, K, H

Corresponding modifications may be performed in corresponding positions of other RP-II proteases.

Substitution with proline residues

Improved thermostability of a RP-II protease can be obtained by subjecting the RP-II protease in question to analysis for secondary structure, identifying residues in the RP-II protease having dihedral angles ϕ (phi) and ψ (psi) confined to the intervals [-90°<ϕ<-40° and -180°<ψ<180°], preferably the intervals [-90°<ϕ<-40° and 120°<ψ<180°] or [-90°<ϕ<-40° and -50°<ψ<10°] and excluding residues located in regions in which the RP-II protease is characterized by possessing α-helical or β-sheet structure.

After the dihedral angles ϕ (phi) and ψ (psi) for the amino acids have been calculated, based on the atomic structure in the crystalline RP-II proteases, it is possible to select position(s) which has/have dihedral phi and psi angles favourable for substitution with a proline residue. The aliphatic side chain of proline residues is bonded covalently to the nitrogen atom of the peptide group. The resulting cyclic five-membered ring consequently imposes a rigid constraint on the rotation about the N-C_α bond of the peptide backbone

and simultaneously prevents the formation of hydrogen bonding to the backbone N-atom.

For these structural reasons, proline residues are generally not compatible with α-helical and β-sheet secondary conformations.

If a proline residue is not already at the identified position(s), the naturally occurring amino acid residue is substituted with a proline residue, preferably by site directed mutagenesis applied on a gene encoding the RP-II protease in question.

In the group of BLC- like proteases proline residues can be introduced at positions 18, 115, 185, 269 and 293. Accordingly, a preferred BLC variant has one or more of the substitutions: T60P, S221P, G193P, and V194P.

Alteration of activity:

Amino acid residues at a distance of less than 10Å from the active site residues are most likely to influence the specificity and activity of the RP-II proteases, therefore variants comprising modifications in positions 1, 8, 22-35 (22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35), 42-58 (42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58), 82-100 (82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100), 129-135 (1129, 130, 131,132, 133, 134, 135), 141-142, 153-156 (153, 154, 155, 156), 158, 161-171 (161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171), 188-193 (188, 189, 190, 191, 192, 193), 195,, 201-207 (201, 202, 203, 204, 205, 206, 207), 210, 213-214, 217 may provide a change in activity and/or specificity of the RP-II protease variant.

Substrate binding site

The substrate binding site is identified by the residues in contact with a substrate model, such as the DAFE. The 3D structure coordinates of the BLC protease with DAFE bound in the active site can be found in Appendix 1. Without being limited to any theory, it is presently believed that binding between a substrate and an enzyme is supported by favorable interactions found within a sphere 10 Å from the substrate molecule, in particular within a sphere of 6 Å from the substrate molecule. Examples of such favorable bonds are hydrogen bonds, strong electrostatic interaction and/or hydrophobic interactions.

The following residues of the BLC protease (SEQ ID NO:1), are within a distance of 10Å from the peptide DAFE and thus believed to be involved in interactions with said substrate: 1, 2, 3, 8, 25, 29, 30, 31, 32, 33, 34, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53,

90, 91, 92, 93, 94, 95, 96, 97, 129, 131, 132, 133, 134, 135, 155, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 171, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 200 and 204.

The following residues of the BLC protease (SEQ ID NO: 1), are within a distance of 6Å from the peptide DAFE and thus believed to be involved in interactions with said substrate: 1, 2, 31, 32, 47, 48, 88, 91, 93, 96, 162, 163, 164, 165, 166, 167, 168, 190, 191, 192, 193, 194, 195, and 201.

Helix capping:

For the RP-II proteases helix capping may be obtained by modifying the position structurally corresponding to position 221 in BLC, and specifically in BLC by the modification A221N,T

Removal of deamidation sites

For the RP-II proteases, removal of deamidation sites may be obtained by modifying the positions structurally corresponding to positions 213, 216, and 222 of BLC, and specifically in BLC by the modifications.

N213A,C,D,E,F,G,H,I,K,L,P,Q,R,S,T,V,Y,M,W preferably N213L,T,S

N216A,C,D,E,F,G,H,I,K,L,P,Q,R,S,T,V,Y,M,W preferably N216L,T,S

N222A,C,D,E,F,G,H,I,K,L,P,Q,R,S,T,V,Y,M,W preferably N222L,T,S

Combined modifications

The present invention also encompasses any of the above mentioned RP-II protease variants in combination with any other modification to the amino acid sequence thereof. Especially combinations with other modifications known in the art to provide improved properties to the enzyme are envisaged. Such modifications to be combined with any of the above indicated modifications are exemplified in the following.

Removal of critical oxidation sites

In order to increase the stability of the RP-II protease it may be advantageous to substitute or delete critical oxidation sites, such as methionines, with other amino acid residues which are not subject to oxidation.

Accordingly, in a further embodiment the present invention relates to an RP-II protease variant, in which one or more amino acid residues susceptible to oxidation, especially methionine residues exposed to the surface of the molecule, is/are deleted or replaced with another amino acid residue less susceptible to oxidation. The amino acid residue less susceptible to oxidation may for instance be selected from the group consisting of A, E, N, Q, I, L, S and K.

Specific such variants comprises at least one of the deletions or substitutions M36{*,S,A,N,Q,K}; M160{*,S,A,N,Q,K} of the BLC protease; M144{*,S,A,N,Q,K} of the AC116 and CDJ31 proteases; M67{*,S,A,N,Q,K}, M79{*,S,A,N,Q,K}, M137{*,S,A,N,Q,K}, M144{*,S,A,N,Q,K}, and M171{*,S,A,N,Q,K} of the BO32, BIP and JA96 proteases; M159{*,S,A,N,Q,K} of the BO32 protease; M81{*,S,A,N,Q,K}, and M141{*,S,A,N,Q,K} in the MPR protease; and M17{*,S,A,N,Q,K}, M67{*,S,A,N,Q,K}, M144{*,S,A,N,Q,K}, M160{*,S,A,N,Q,K}, M186{*,S,A,N,Q,K}, and M217{*,S,A,N,Q,K} of the AA513 protease (positions are indicated in relation to the BLC protease as indicated in Fig. 2).

Modification of Asn-Gly sequences in the protease

It is known that at alkaline pH, the side chain of Asn may interact with the NH group of a sequential neighboring amino acid to form an isoAsp residue where the backbone goes through the Asp side chain. This will leave the backbone more vulnerable to proteolysis. The deamidation is much more likely to occur if the residue that follows is a Gly. Changing the Asn in front of the Gly or the Gly will prevent this from happening and thus improve the stability, especially as concerns thermo- and storage stability.

The invention consequently further relates to an RP-II protease variant, in which either or both residues of any of the Asn-Gly sequence appearing in the amino acid sequence of the parent RP-II protease is/are deleted or substituted with a residue of a different amino acid.

The Asn and/or Gly residue may, for instance, be substituted with a residue of an amino acid selected from the group consisting of A, Q, S, P, T and Y.

More specifically, any of the Asn or Gly residues of the Asn-Gly occupying positions 68-69, 182-183 and/or 192-193 of the BLC protease; positions 68-69 and/or 192-193 of the AC116 and CDJ-31 proteases, positions 45-46, 74-75, 196-197, and/or

201-202 of the BO32, JA96 and BIP proteases, positions 68-69, 103-104 and/or 192-196 of the MPR protease; and positions 90-91 and/or 201-202 of the AA513 protease, may be deleted or substituted with a residue of an amino acid selected from the group consisting of A, Q, S, P, T and Y. (positions are indicated in relation to the BLC protease as indicated in Fig. 2)

Specific variants of BLC are:

N68{*,A,Q,S,P,T,Y}; G69{*,A,Q,S,P,T,Y}

N68{*,A,Q,S,P,T,Y}+G69{*,A,Q,S,P,T,Y}

N182{*,A,Q,S,P,T,Y}; G183{*,A,Q,S,P,T,Y}

N182{*,A,Q,S,P,T,Y}+G183{*,A,Q,S,P,T,Y}

N192{*,A,Q,S,P,T,Y}; G193{*,A,Q,S,P,T,Y}

N192{*,A,Q,S,P,T,Y}+G193{*,A,Q,S,P,T,Y}

and combinations thereof.

Specific variants of the AC116 and CDJ-31 proteases are:

N68{*,A,Q,S,P,T,Y}; G69{*,A,Q,S,P,T,Y}

N68{*,A,Q,S,P,T,Y}+G69{*,A,Q,S,P,T,Y}

N192{*,A,Q,S,P,T,Y}; G193{*,A,Q,S,P,T,Y}

N192{*,A,Q,S,P,T,Y}+G193{*,A,Q,S,P,T,Y}

N68{*,A,Q,S,P,T,Y}+N192{*,A,Q,S,P,T,Y}

and combinations thereof.

Specific variants of BO32, JA96 and BIP proteases are:

N45{*,A,Q,S,P,T,Y}; G46{*,A,Q,S,P,T,Y}

N45{*,A,Q,S,P,T,Y}+G46{*,A,Q,S,P,T,Y}

N74{*,A,Q,S,P,T,Y}; G75{*,A,Q,S,P,T,Y}

N74{*,A,Q,S,P,T,Y}+G75{*,A,Q,S,P,T,Y}

N196{*,A,Q,S,P,T,Y}; G197{*,A,Q,S,P,T,Y}

N196{*,A,Q,S,P,T,Y}+G197{*,A,Q,S,P,T,Y}

N201{*,A,Q,S,P,T,Y}; G202{*,A,Q,S,P,T,Y}

N201{*,A,Q,S,P,T,Y} + G202{*,A,Q,S,P,T,Y}

N45{*,A,Q,S,P,T,Y}+N74{*,A,Q,S,P,T,Y}

N45{*,A,Q,S,P,T,Y}+N196{*,A,Q,S,P,T,Y}

N45{*,A,Q,S,P,T,Y}+N201{*,A,Q,S,P,T,Y}

N74{*,A,Q,S,P,T,Y}+N196{*,A,Q,S,P,T,Y}

N74{*,A,Q,S,P,T,Y}+N201{*,A,Q,S,P,T,Y}

N196{*,A,Q,S,P,T,Y}+N201{*,A,Q,S,P,T,Y}

N45{*,A,Q,S,P,T,Y}+N74{*,A,Q,S,P,T,Y}+N196{*,A,Q,S,P,T,Y}

N45{*,A,Q,S,P,T,Y}+N74{*,A,Q,S,P,T,Y}+N201{*,A,Q,S,P,T,Y}

N45{*,A,Q,S,P,T,Y}+N196{*,A,Q,S,P,T,Y}+N201{*,A,Q,S,P,T,Y}

N74{*,A,Q,S,P,T,Y}+N196{*,A,Q,S,P,T,Y}+N201{*,A,Q,S,P,T,Y}

N45{*,A,Q,S,P,T,Y}+N74{*,A,Q,S,P,T,Y}+N196{*,A,Q,S,P,T,Y}+N201{*,A,Q,S,P,T,Y}

and combinations thereof.

Specific variants of AA513 are:

N90{*,A,Q,S,P,T,Y}; G91{*,A,Q,S,P,T,Y}

N90{*,A,Q,S,P,T,Y}+G91{*,A,Q,S,P,T,Y}

N201{*,A,Q,S,P,T,Y}; G202{*,A,Q,S,P,T,Y}

N201{*,A,Q,S,P,T,Y}+G202{*,A,Q,S,P,T,Y}

N90{*,A,Q,S,P,T,Y}+N201{*,A,Q,S,P,T,Y}

and combinations thereof.

Specific variants of MPR are:

N68{*,A,Q,S,P,T,Y}; G69{*,A,Q,S,P,T,Y}

N68{*,A,Q,S,P,T,Y}+G69{*,A,Q,S,P,T,Y}

N103{*,A,Q,S,P,T,Y}; G104{*,A,Q,S,P,T,Y}

N103{*,A,Q,S,P,T,Y}+G104{*,A,Q,S,P,T,Y}

N192{*,A,Q,S,P,T,Y}; G196{*,A,Q,S,P,T,Y}

N192{*,A,Q,S,P,T,Y}+G196{*,A,Q,S,P,T,Y}

N68{*,A,Q,S,P,T,Y}+N103{*,A,Q,S,P,T,Y}

N68{*,A,Q,S,P,T,Y}+N192{*,A,Q,S,P,T,Y}

N103{*,A,Q,S,P,T,Y}+N192{*,A,Q,S,P,T,Y}

N68{*,A,Q,S,P,T,Y}+N103{*,A,Q,S,P,T,Y}+N192{*,A,Q,S,P,T,Y}

and combinations thereof.

Removal of autoproteolysis sites

According to a further aspect of the invention autoproteolysis sites may be removed by changing the amino acids at an autoproteolysis site. Since the RP-II proteases cleaves at Glu and Asp residues it is preferred to modify such residues of a parent RP-II protease having the same or a similar specificity, preferably by substituting with any other amino acid except Glu.

The parent RP-II proteases are mostly specific towards Glu and to a minor extent towards Asp residues. Therefore the modification of the parent (trypsin-like) RP-II protease may preferably be made by changing Glu to another amino acid residue (including Asp). Experiments have indicated that the substitution of Ala for Glu or Asp

provides good results.

Glu and Asp residue are in the BLC, CDJ31 and AC116 proteases found in positions E101, ,E152, E173, E209, D6, D51, D96, D135, D161, and D212. BLC has a further Glu in position E104 and Asp in D7.

Specific BLC, CDJ31 and AC116 variants are thus E101A, E152A, E173A, E209A, D6A, D51A, D135A, D161A, D212A, and double, triple, quadruple, etc. combinations thereof. Further specific BLC variants are E104A and D7A.

In JA96, BO32 and BIP Glu and Asp are found at positions E81, E143, E151, E209, D5, D6, D69, D96, D103, D135, D152, D161, and D173.

Specific JA96, BO32 and BIP variants are thus E81A, E143A, E151A, E202A, D5A, D6A, D69A, D96A, D103A, D135A, D152A, D161A, D173A, and double, triple, quadruple, etc. combinations thereof.

In MPR Glu and Asp are found at positions E7, E89a, E152, D6, D54, D92, D96, D135, D144, D161, D177 and D209

Specific MPR variants are thus E7A, E89aA, E152A, D6A, D54A, D92A, D96A, D135A, D144A, D161A, D177A and D209A, and double, triple, quadruple, etc. combinations thereof.

In AA513 Glu and Asp are found at positions E26, E55, E94, E117, E123, E137b, E199, D40, D96, D103b, D103d, D135, D149, D154, D161, D184 and D209

Specific AA513 variants are thus E26A, E55A, E94A, E117A, E123A, E137bA, E199A, D40A, D96A, D103bA, D103dA, D135A, D149A, D154A, D161A, D184A and D209A, and double, triple, quadruple, etc. combinations thereof.

Corresponding variants are easily identified in any other RP-II protease.

Alternatively autoproteolysis can be prevented by changing the amino acid residue occupying the 1st and/or 2nd position following the Glu or Asp residue in question to Pro. For instance, this may in BLC, CDJ31 and AC116 be done in the positions 174 and/or 175 as follows:

Q174P; S175P; Q174P+S175P

or in a similar manner in JA96, BO32 or BIP at positions 152 and/or 153 as D152P; T153P; or D152P+T153P.

Corresponding variants are easily identified in these and any other RP-II protease.

Modification of tryptophan residues

In order to stabilize the protein it may be advantageous to replace or delete tryptophan residues at the surface of the protein, *e.g.*, as described in US 5,118,623. The tryptophan residues may advantageously be substituted for F, T, Q or G. Thus, in a further embodiment the invention relates to an RP-II variant comprising one or more of the following substitutions:

BLC and AC116:

W35{F,T,Q,G}; W88{F,T,Q,G}; W142{F,T,Q,G}; W217{F,T,Q,G}

CDJ31:

W142{F,T,Q,G}; W217{F,T,Q,G};

BO32, JA96 and BIP:

W142{F,T,Q,G};

AA513:

W30{F,T,Q,G}; W72{F,T,Q,G}; W142{F,T,Q,G}

MPR:

W57{F,T,Q,G}; W88{F,T,Q,G}; W112{F,T,Q,G}; W142{F,T,Q,G}; W217{F,T,Q,G}

Modification of tyrosines

In relation to wash performance it has been found that the modification of certain tyrosine residues to phenylalanine provides an improved wash performance. Without being bound by any specific theory, it is believed that titration of these Tyr residues in the alkaline wash liquor has negative effects that are alleviated by replacing the Tyr residues with other residues, especially Phe or Trp, particularly Phe.

In the BLC, AC116 and CDJ31 parent RP-II proteases, the following tyrosine

residues may be modified:

19, 50, 72, 74, 82, 95, 97, 112, 115, 117, 132, 154, 163, 195, 200. In BLC and CDJ31 the tyrosines in positions 17 and 158 may also be modified , and in AC116 and CDJ31 the tyrosines in position 172

Examples of specific variants comprise one or more of the following substitutions:

Y17{F,W}, Y19{F,W}, Y50{F,W}, Y72{F,W}, Y74{F,W}, Y82{F,W}, Y88{F,W}, Y95{F,W}, Y97{F,W}, Y112{F,W}, Y115{F,W}, Y117{F,W}, Y132{F,W}, Y154{F,W}, Y158{F,W}, Y163{F,W}, Y172{F,W}, Y195{F,W}, Y200{F,W}

In the JA96, BO32 and BIP parent RP-II proteases, the following tyrosine residues may be modified:

19, 24, 50, 57, 64, 83, 88, 95, 112, 132, 157, 158, 195, 216

Examples of specific JA96, BO32 and BIP variants comprises one or more of the following substitutions:

Y19{F,W}, Y24{F,W}, Y50{F,W}, Y57{F,W}, Y64{F,W}, Y83{F,W}, Y88{F,W}, Y95{F,W}, Y112{F,W}, Y132{F,W}, Y157{F,W}, Y158{F,W}, Y195{F,W} and Y216{F,W}

In the AA513 parent RP-II protease, the following tyrosine residues may be modified:

24, 74, 77, 84, 88, 97, 130, 132, 158, 163, 193a

Examples of specific AA513 variants comprises one or more of the following substitutions:

Y24{F,W}, Y74{F,W}, Y77{F,W}, Y84{F,W}, Y88{F,W}, Y97{F,W}, Y130{F,W}, Y132{F,W}, Y158{F,W}, Y163{F,W}, Y193A{F,W}

In the MPR parent RP-II protease, the following tyrosine residues may be modified:

19, 28a, 30, 50, 72, 74, 77, 83, 95, 97, 113, 115, 154, 158, 163, 172, 175, 200, 216

Examples of specific MPR variants comprises one or more of the following sub-

stitutions:

Y19{F,W}, Y28Ad{F,W}, Y30{F,W}, Y50{F,W}, Y72{F,W}, Y74{F,W}, Y77{F,W}, Y83{F,W}, Y95{F,W}, Y97{F,W}, Y113{F,W}, 115{F,W}, Y154{F,W}, Y158{F,W}, Y163{F,W}, Y172{F,W}, Y175{F,W}, Y200{F,W}, Y216{F,W}

Other modifications for combination

Examples of specific BLC variants comprises one or more of the following substitutions:

E152{A,R,K,G}

E173A

E209A

E152G+G164R

METHODS OF PREPARING RP-II PROTEASE VARIANTS

The RP-II protease variants of the present invention may be produced by any known method within the art. The invention also relates to polynucleotides encoding the RP-II protease variants of the present invention, DNA constructs comprising such polynucleotides and host cells comprising such constructs or polynucleotides.

In general natural occurring proteins may be produced by culturing the organism expressing the protein and subsequently purifying the protein, or recombinantly by cloning a polynucleotide, e.g. genomic DNA or cDNA, encoding the protein into an expression vector, introducing said expression vector into a host cell, culturing the host cell and purifying the expressed protein.

site-directed mutagenesis

Typically protein variants may be produced by site-directed mutagenesis of the gene encoding a parent protein, introduction of the mutated gene into an expression vector, host cell etc. The gene encoding the parent protein may be cloned from a strain producing the polypeptide or from an expression library, i.e. it may be isolated from ge-

nomic DNA or prepared from cDNA, or a combination thereof. The gene may even be a fully synthetically produced gene.

In general standard procedures for cloning of genes and/or introducing mutations (random and/or site directed) into said genes may be used in order to obtain a parent RP-II protease, or RP-II protease variant of the invention. For further description of suitable techniques reference is made to Molecular cloning: A laboratory manual (Sambrook et al. (1989), Cold Spring Harbor lab., Cold Spring Harbor, NY; Ausubel, F. M. et al. (eds.)); Current protocols in Molecular Biology (John Wiley and Sons, 1995; Harwood, C. R., and Cutting, S. M. (eds.)); Molecular Biological Methods for Bacillus (John Wiley and Sons, 1990); DNA Cloning: A Practical Approach, Volumes I and II (D.N. Glover ed. 1985); Oligonucleotide Synthesis (M.J. Gait ed. 1984); Nucleic Acid Hybridization (B.D. Hames & S.J. Higgins eds (1985)); Transcription And Translation (B.D. Hames & S.J. Higgins, eds. (1984)); Animal Cell Culture (R.I. Freshney, ed. (1986)); Immobilized Cells And Enzymes (IRL Press, (1986)); A Practical Guide To Molecular Cloning (B. Perbal, (1984)) and WO 96/34946.

Localized and region specific random mutagenesis

Random mutagenesis is suitably performed either as localized or region-specific random mutagenesis in at least three parts of the gene translating to the amino acid sequence shown in question, or within the whole gene.

The random mutagenesis of a DNA sequence encoding a parent RP-II protease may be conveniently performed by use of any method known in the art.

In relation to the above, a further aspect of the present invention relates to a method for generating a variant of a parent RP-II protease wherein the variant exhibits an altered property, such as increased thermostability, increased stability at low pH and at low calcium concentration, relative to the parent RP-II protease, the method comprising:

a) subjecting a DNA sequence encoding the parent protease to localized or region-specific random mutagenesis,

b) expressing the mutated DNA sequence obtained in step (a) in a host cell, and

c) screening for host cells expressing a RP-II protease variant which has an altered property relative to the parent RP-II protease.

Step (a) of the above method of the invention is preferably performed using doped primers.

When the mutagenesis is performed by the use of an oligonucleotide, the oligonucleotide may be doped or spiked with the three non-parent nucleotides during the synthesis of the oligonucleotide at the positions that are to be changed. The doping or spiking may be done so that codons for unwanted amino acids are avoided. The doped or spiked oligonucleotide can be incorporated into the DNA encoding the RP-II protease by any published technique, using, e.g., PCR, LCR or any DNA polymerase and ligase as deemed appropriate.

Preferably, the doping is carried out using "constant random doping", in which the percentage of wild-type and modification in each position is predefined. Furthermore, the doping may be directed toward a preference for the introduction of certain nucleotides, and thereby a preference for the introduction of one or more specific amino acid residues. The doping may be made, e.g., so as to allow for the introduction of 90% wild type and 10% modifications in each position. An additional consideration in the choice of a doping scheme is based on genetic as well as protein-structural constraints. The doping scheme may be made by using the DOPE program which, *inter alia*, ensures that introduction of stop codons is avoided (L.J. Jensen et al. *Nucleic Acid Research*, 26, 697-702 (1998).

The DNA sequence to be mutagenized may conveniently be present in a genomic or cDNA library prepared from an organism expressing the parent RP-II protease. Alternatively, the DNA sequence may be present on a suitable vector such as a plasmid or a bacteriophage, which as such may be incubated with or otherwise exposed to the mutagenizing agent. The DNA to be mutagenized may also be present in a host cell either by being integrated in the genome of said cell or by being present on a vector harboured in the cell. Finally, the DNA to be mutagenized may be in isolated form. It will be understood that the DNA sequence to be subjected to random mutagenesis is preferably a cDNA or a genomic DNA sequence.

In some cases it may be convenient to amplify the mutated DNA sequence prior to performing the expression step b) or the screening step c). Such amplification may be performed in accordance with methods known in the art, the presently preferred method being PCR-generated amplification using oligonucleotide primers prepared on the basis of the DNA or amino acid sequence of the parent enzyme.

Subsequent to the incubation with or exposure to the mutagenizing agent, the mutated DNA is expressed by culturing a suitable host cell carrying the DNA sequence under conditions allowing expression to take place. The host cell used for this purpose may be one which has been transformed with the mutated DNA sequence, optionally

present on a vector, or one which was carried the DNA sequence encoding the parent enzyme during the mutagenesis treatment. Examples of suitable host cells are the following: gram positive bacteria such as *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus lentus*, *Bacillus brevis*, *Bacillus stearothermophilus*, *Bacillus alkalophilus*, *Bacillus amyloliquefaciens*, *Bacillus coagulants*, *Bacillus circulans*, *Bacillus lautus*, *Bacillus megaterium*, *Bacillus thuringiensis*, *Streptomyces lividans* or *Streptomyces murinus*; and gram negative bacteria such as *E. coli*.

The mutated DNA sequence may further comprise a DNA sequence encoding functions permitting expression of the mutated DNA sequence.

Localised random mutagenesis

The random mutagenesis may be advantageously localised to a part of the parent RP-II protease in question. This may, e.g., be advantageous when certain regions of the enzyme have been identified to be of particular importance for a given property of the enzyme, and when modified are expected to result in a variant having improved properties. Such regions may normally be identified when the tertiary structure of the parent enzyme has been elucidated and related to the function of the enzyme.

The localised or region-specific, random mutagenesis is conveniently performed by use of PCR generated mutagenesis techniques as described above or any other suitable technique known in the art. Alternatively, the DNA sequence encoding the part of the DNA sequence to be modified may be isolated, e.g., by insertion into a suitable vector, and said part may be subsequently subjected to mutagenesis by use of any of the mutagenesis methods discussed above.

<u>General method for localised random mutagenesis by use of the DOPE program</u>

The localised random mutagenesis may be carried out by the following steps:

1. Select regions of interest for modification in the parent enzyme
2. Decide on mutation sites and non-mutated sites in the selected region
3. Decide on which kind of mutations should be carried out, e.g. with respect to the desired stability and/or performance of the variant to be constructed
4. Select structurally based mutations
5. Adjust the residues selected in step 3 with regard to step 4.
6. Analyse by use of a suitable dope algorithm the nucleotide distribu-

tion.

7. If necessary, adjust the wanted residues to genetic code realism, e.g. taking into account constraints resulting from the genetic code, e.g. in order to avoid introduction of stop codons; the skilled person will be aware that some codon combinations cannot be used in practice and will need to be adapted
8. Make primers
9. Perform localised random mutagenesis by use of the primers
10. Select resulting RP-II protease variants by screening for the desired improved properties.

Suitable dope algorithms for use in step 6 are well known in the art. One such algorithm is described by Tomandl, D. et al., 1997, Journal of Computer-Aided Molecular Design 11:29-38. Another algorithm is DOPE (Jensen, LJ, Andersen, KV, Svendsen, A, and Kretzschmar, T (1998) Nucleic Acids Research 26:697-702).

Expression vectors

A recombinant expression vector comprising a nucleic acid sequence encoding a RP-II protease variant of the invention may be any vector that may conveniently be subjected to recombinant DNA procedures and which may bring about the expression of the nucleic acid sequence.

The choice of vector will often depend on the host cell into which it is to be introduced. Examples of a suitable vector include a linear or closed circular plasmid or a virus. The vector may be an autonomously replicating vector, i.e., a vector which exists as an extra-chromosomal entity, the replication of which is independent of chromosomal replication, e.g., a plasmid, an extra-chromosomal element, a mini chromosome, or an artificial chromosome. The vector may contain any means for assuring self-replication. Examples of bacterial origins of replication are the origins of replication of plasmids pBR322, pUC19, pACYC177, pACYC184, pUB110, pE194, pTA1060, and pAMß1. Examples of origin of replications for use in a yeast host cell are the 2 micron origin of replication, the combination of CEN6 and ARS4, and the combination of CEN3 and ARS1. The origin of replication may be one having a mutation which makes it function as temperature-sensitive in the host cell (see, e.g., Ehrlich, 1978, Proceedings of the National Academy of Sciences USA 75:1433).

Alternatively, the vector may be one which, when introduced into the host cell, is integrated into the genome and replicated together with the chromosome(s) into which it has been integrated. Vectors which are integrated into the genome of the host cell may contain any nucleic acid sequence enabling integration into the genome; in particular it may contain nucleic acid sequences facilitating integration into the genome by homologous or non-homologous recombination. The vector system may be a single vector, e.g. plasmid or virus, or two or more vectors, e.g. plasmids or virus', which together contain the total DNA to be introduced into the genome of the host cell, or a transposon.

The vector may in particular be an expression vector in which the DNA sequence encoding the RP-II protease variant of the invention is operably linked to additional segments or control sequences required for transcription of the DNA. The term, "operably linked" indicates that the segments are arranged so that they function in concert for their intended purposes, e.g. transcription initiates in a promoter and proceeds through the DNA sequence encoding the RP-II protease variant. Additional segments or control sequences include a promoter, a polyadenylation sequence, a propeptide sequence, a signal sequence and a transcription terminator. At a minimum the control sequences include a promoter and transcriptional and translational stop signals.

The promoter may be any DNA sequence that shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell.

Examples of suitable promoters for use in bacterial host cells include the promoter of the *Bacillus subtilis* levansucrase gene (sacB), the *Bacillus stearothermophilus* maltogenic amylase gene (amyM), the *Bacillus licheniformis* alpha-amylase gene (amyL), the *Bacillus amyloliquefaciens* alpha-amylase gene (amyQ), the *Bacillus subtilis* alkaline protease gene, or the *Bacillus pumilus* xylosidase gene, the *Bacillus amyloliquefaciens* BAN amylase gene, the *Bacillus licheniformis* penicillinase gene (penP), the *Bacillus subtilis* xylA and xylB genes, and the prokaryotic beta-lactamase gene (Villa-Kamaroff et al., 1978, Proceedings of the National Academy of Sciences USA 75:3727-3731). Other examples include the phage Lambda P_R or P_L promoters or the E. coli lac, trp or tac promoters or the Streptomyces coelicolor agarase gene (dagA). Further promoters are described in "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242:74-94; and in Sambrook et al., 1989, supra.

Examples of suitable promoters for use in a filamentous fungal host cell are promoters obtained from the genes encoding *Aspergillus oryzae* TAKA amylase, *Rhi-*

zomucor miehei aspartic proteinase, *Aspergillus niger* neutral alpha-amylase, *Aspergillus niger* acid stable alpha-amylase, *Aspergillus niger* or *Aspergillus awamori* glucoamylase (glaA), *Rhizomucor miehei* lipase, *Aspergillus oryzae* alkaline protease, *Aspergillus oryzae* triose phosphate isomerase, *Aspergillus nidulans* acetamidase, *Fusarium oxysporum* trypsin-like protease (as described in U.S. Patent No. 4,288,627, which is incorporated herein by reference), and hybrids thereof. Particularly preferred promoters for use in filamentous fungal host cells are the TAKA amylase, NA2-tpi (a hybrid of the promoters from the genes encoding *Aspergillus niger* neutral (-amylase and *Aspergillus oryzae* triose phosphate isomerase), and glaA promoters. Further suitable promoters for use in filamentous fungus host cells are the ADH3 promoter (McKnight et al., The EMBO J. 4 (1985), 2093 - 2099) or the tpiA promoter.

Examples of suitable promoters for use in yeast host cells include promoters from yeast glycolytic genes (Hitzeman et al., J. Biol. Chem. 255 (1980), 12073 - 12080; Alber and Kawasaki, J. Mol. Appl. Gen. 1 (1982), 419 - 434) or alcohol dehydrogenase genes (Young et al., in Genetic Engineering of Microorganisms for Chemicals (Hollaender et al, eds.), Plenum Press, New York, 1982), or the TPI1 (US 4,599,311) or ADH2-4c (Russell et al., Nature 304 (1983), 652 - 654) promoters.

Further useful promoters are obtained from the *Saccharomyces cerevisiae* enolase (ENO-1) gene, the *Saccharomyces cerevisiae* galactokinase gene (GAL1), the *Saccharomyces cerevisiae* alcohol dehydrogenase/glyceraldehyde-3-phosphate dehydrogenase genes (ADH2/GAP), and the *Saccharomyces cerevisiae* 3-phosphoglycerate kinase gene. Other useful promoters for yeast host cells are described by Romanos et al., 1992, Yeast 8:423-488. In a mammalian host cell, useful promoters include viral promoters such as those from Simian Virus 40 (SV40), Rous sarcoma virus (RSV), adenovirus, and bovine papilloma virus (BPV).

Examples of suitable promoters for use in mammalian cells are the SV40 promoter (Subramani et al., Mol. Cell Biol. 1 (1981), 854 -864), the MT-1 (metallothionein gene) promoter (Palmiter et al., Science 222 (1983), 809 - 814) or the adenovirus 2 major late promoter.

An example of a suitable promoter for use in insect cells is the polyhedrin promoter (US 4,745,051; Vasuvedan et al., FEBS Lett. 311, (1992) 7 - 11), the P10 promoter (J.M. Vlak et al., J. Gen. Virology 69, 1988, pp. 765-776), the Autographa californica polyhedrosis virus basic protein promoter (EP 397 485), the baculovirus immediate early gene 1 promoter (US 5,155,037; US 5,162,222), or the baculovirus 39K delayed-early gene promoter (US 5,155,037; US 5,162,222).

The DNA sequence encoding a RP-II protease variant of the invention may also, if necessary, be operably connected to a suitable terminator.

The recombinant vector of the invention may further comprise a DNA sequence enabling the vector to replicate in the host cell in question.

The vector may also comprise a selectable marker, e.g. a gene the product of which complements a defect in the host cell, or a gene encoding resistance to e.g. antibiotics like ampicillin, kanamycin, chloramphenicol, erythromycin, tetracycline, spectinomycine, neomycin, hygromycin, methotrexate, or resistance to heavy metals, virus or herbicides, or which provides for prototrophy or auxotrophs. Examples of bacterial selectable markers are the dal genes from *Bacillus subtilis* or *Bacillus licheniformis*, resistance. A frequently used mammalian marker is the dihydrofolate reductase gene (DHFR). Suitable markers for yeast host cells are ADE2, HIS3, LEU2, LYS2, MET3, TRP1, and URA3. A selectable marker for use in a filamentous fungal host cell may be selected from the group including, but not limited to, amdS (acetamidase), argB (ornithine carbamoyltransferase), bar (phosphinothricin acetyltransferase), hygB (hygromycin phosphotransferase), niaD (nitrate reductase), pyrG (orotidine-5'-phosphate decarboxylase), sC (sulfate adenyltransferase), trpC (anthranilate synthase), and glufosinate resistance markers, as well as equivalents from other species. Particularly, for use in an *Aspergillus* cell are the amdS and pyrG markers of *Aspergillus nidulans* or *Aspergillus oryzae* and the bar marker of *Streptomyces hygroscopicus*. Furthermore, selection may be accomplished by co-transformation, e.g., as described in WO 91/17243, where the selectable marker is on a separate vector.

To direct a RP-II protease variant of the present invention into the secretory pathway of the host cells, a secretory signal sequence (also known as a leader sequence, prepro sequence or pre sequence) may be provided in the recombinant vector. The secretory signal sequence is joined to the DNA sequence encoding the enzyme in the correct reading frame. Secretory signal sequences are commonly positioned 5' to the DNA sequence encoding the enzyme. The secretory signal sequence may be that normally associated with the enzyme or may be from a gene encoding another secreted protein.

The procedures used to ligate the DNA sequences coding for the present enzyme, the promoter and optionally the terminator and/or secretory signal sequence, respectively, or to assemble these sequences by suitable PCR amplification schemes, and to insert them into suitable vectors containing the information necessary for replication or integration, are well known to persons skilled in the art (cf., for instance, Sam-

brook et al.).

More than one copy of a nucleic acid sequence encoding an enzyme of the present invention may be inserted into the host cell to amplify expression of the nucleic acid sequence. Stable amplification of the nucleic acid sequence can be obtained by integrating at least one additional copy of the sequence into the host cell genome using methods well known in the art and selecting for transformants.

The nucleic acid constructs of the present invention may also comprise one or more nucleic acid sequences which encode one or more factors that are advantageous in the expression of the polypeptide, e.g., an activator (e.g., a trans-acting factor), a chaperone, and a processing protease. Any factor that is functional in the host cell of choice may be used in the present invention. The nucleic acids encoding one or more of these factors are not necessarily in tandem with the nucleic acid sequence encoding the polypeptide.

Host cells

The DNA sequence encoding a RP-II protease variant of the present invention may be either homologous or heterologous to the host cell into which it is introduced. If homologous to the host cell, i.e. produced by the host cell in nature, it will typically be operably connected to another promoter sequence or, if applicable, another secretory signal sequence and/or terminator sequence than in its natural environment. The term "homologous" is intended to include a DNA sequence encoding an enzyme native to the host organism in question. The term "heterologous" is intended to include a DNA sequence not expressed by the host cell in nature. Thus, the DNA sequence may be from another organism, or it may be a synthetic sequence.

The host cell into which the DNA construct or the recombinant vector of the invention is introduced may be any cell that is capable of producing the present RP-II protease variants, such as prokaryotes, e.g. bacteria or eukaryotes, such as fungal cells, e.g. yeasts or filamentous fungi, insect cells, plant cells or mammalian cells.

Examples of bacterial host cells which, on cultivation, are capable of producing the RP-II protease variants of the invention are gram-positive bacteria such as strains of *Bacillus*, e.g. strains of *B. subtilis*, *B. licheniformis*, *B. lentus*, *B. brevis*, *B. stearothermophilus*, *B. alkalophilus*, *B. amyloliquefaciens*, *B. coagulans*, *B. circulans*, *B. lautus*, *B. megaterium* or *B. thuringiensis*, or strains of *Streptomyces*, such as *S. lividans* or *S. murinus*, or gram-negative bacteria such as *Escherichia coli* or *Pseudomo-*

nas sp.

The transformation of the bacteria may be effected by protoplast transformation, electroporation, conjugation, or by using competent cells in a manner known per se (cf. Sambrook et al., supra).

When expressing the RP-II protease variant in bacteria such as *E. coli*, the enzyme may be retained in the cytoplasm, typically as insoluble granules (known as inclusion bodies), or it may be directed to the periplasmic space by a bacterial secretion sequence. In the former case, the cells are lysed and the granules are recovered and denatured after which the enzyme is refolded by diluting the denaturing agent. In the latter case, the enzyme may be recovered from the periplasmic space by disrupting the cells, e.g. by sonication or osmotic shock, to release the contents of the periplasmic space and recovering the enzyme.

When expressing the RP-II protease variant in gram-positive bacteria such as *Bacillus* or *Streptomyces* strains, the enzyme may be retained in the cytoplasm, or it may be directed to the extracellular medium by a bacterial secretion sequence. In the latter case, the enzyme may be recovered from the medium as described below.

Examples of host yeast cells include cells of a species of *Candida*, *Kluyveromyces*, *Saccharomyces*, *Schizosaccharomyces*, *Pichia*, *Hansehula*, or *Yarrowia*. In a particular embodiment, the yeast host cell is a *Saccharomyces carlsbergensis*, *Saccharomyces cerevisiae*, *Saccharomyces diastaticus*, *Saccharomyces douglasii*, *Saccharomyces kluyveri*, *Saccharomyces norbensis* or *Saccharomyces oviformis* cell. Other useful yeast host cells are a *Kluyveromyces lactis*, *Kluyveromyces fragilis*, *Hansehula polymorpha*, *Pichia pastoris*, *Yarrowia lipolytica*, *Schizosaccharomyces pombe*, *Ustilgo maylis*, *Candida maltose*, *Pichia guillermondii* and *Pichia methanolio* cell (cf. Gleeson et al., J. Gen. Microbiol. 132, 1986, pp. 3459-3465; US 4,882,279 and US 4,879,231). Since the classification of yeast may change in the future, for the purposes of this invention, yeast shall be defined as described in Biology and Activities of Yeast (Skinner, F.A., Passmore, S.M., and Davenport, R.R., eds, Soc. App. Bacteriol. Symposium Series No. 9, 1980. The biology of yeast and manipulation of yeast genetics are well known in the art (see, e.g., Biochemistry and Genetics of Yeast, Bacil, M., Horecker, B.J., and Stopani, A.O.M., editors, 2nd edition, 1987; The Yeasts, Rose, A.H., and Harrison, J.S., editors, 2nd edition, 1987; and The Molecular Biology of the Yeast Saccharomyces, Strathern et al., editors, 1981). Yeast may be transformed using the procedures described by Becker and Guarente, In Abelson, J.N. and Simon, M.I., editors, Guide to Yeast Genetics and Molecular Biology, Methods in Enzymology, Volume 194,

pp 182-187, Academic Press, Inc., New York; Ito et al., 1983, Journal of Bacteriology 153:163; and Hinnen et al., 1978, Proceedings of the National Academy of Sciences USA 75:1920.

Examples of filamentous fungal cells include filamentous forms of the subdivision Eumycota and Oomycota (as defined by Hawksworth et al., 1995, supra), in particular it may of the a cell of a species of *Acremonium*, such as *A. chrysogenum*, *Aspergillus*, such as *A. awamori*, *A. foetidus*, *A. japonicus*, *A. niger*, *A. nidulans* or *A. oryzae*, *Fusarium*, such as *F. bactridioides*, *F. cerealis*, *F. crookwellense*, *F. culmorum*, *F. graminearum*, *F. graminum*, *F. heterosporum*, *F. negundi*, *F. reticulatum*, *F. roseum*, *F. sambucinum*, *F. sarcochroum*, *F. sulphureum*, *F. trichothecioides* or *F. oxysporum*, *Humicola*, such as *H. insolens* or *H. lanuginose*, *Mucor*, such as *M. miehei*, *Myceliophthora*, such as *M. thermophilum*, *Neurospora*, such as *N. crassa*, *Penicillium*, such as *P. purpurogenum*, *Thielavia*, such as *T. terrestris*, *Tolypocladium*, or *Trichoderma*, such as *T. harzianum*, *T. koningii*, *T. longibrachiatum*, *T. reesei* or *T. viride*, or a teleomorph or synonym thereof. The use of *Aspergillus spp.* for the expression of proteins is described in, e.g., EP 272 277, EP 230 023.

Examples of insect cells include a *Lepidoptera* cell line, such as *Spodoptera frugiperda* cells or *Trichoplusia ni* cells (cf. US 5,077,214). Culture conditions may suitably be as described in WO 89/01029 or WO 89/01028.Transformation of insect cells and production of heterologous polypeptides therein may be performed as described in US 4,745,051; US 4, 775, 624; US 4,879,236; US 5,155,037; US 5,162,222; EP 397,485).

Examples of mammalian cells include Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, COS cells, or any number of other immortalized cell lines available, e.g., from the American Type Culture Collection. Methods of transfecting mammalian cells and expressing DNA sequences introduced in the cells are described in e.g. Kaufman and Sharp, J. Mol. Biol. 159 (1982), 601 - 621; Southern and Berg, J. Mol. Appl. Genet. 1 (1982), 327 - 341; Loyter et al., Proc. Natl. Acad. Sci. USA 79 (1982), 422 - 426; Wigler et al., Cell 14 (1978), 725; Corsaro and Pearson, Somatic Cell Genetics 7 (1981), 603, Ausubel et al., Current Protocols in Molecular Biology, John Wiley and Sons, Inc., N.Y., 1987, Hawley-Nelson et al., Focus 15 (1993), 73; Ciccarone et al., Focus 15 (1993), 80; Graham and van der Eb, Virology 52 (1973), 456; and Neumann et al., EMBO J. 1 (1982), 841 - 845. Mammalian cells may be transfected by direct uptake using the calcium phosphate precipitation method of Graham and Van der Eb (1978, Virology 52:546).

Methods for expression and isolation of proteins

To express an enzyme of the present invention the above mentioned host cells transformed or transfected with a vector comprising a nucleic acid sequence encoding an enzyme of the present invention are typically cultured in a suitable nutrient medium under conditions permitting the production of the desired molecules, after which these are recovered from the cells, or the culture broth.

The medium used to culture the host cells may be any conventional medium suitable for growing the host cells, such as minimal or complex media containing appropriate supplements. Suitable media are available from commercial suppliers or may be prepared according to published recipes (e.g. in catalogues of the American Type Culture Collection). The media may be prepared using procedures known in the art (see, e.g., references for bacteria and yeast; Bennett, J.W. and LaSure, L., editors, More Gene Manipulations in Fungi, Academic Press, CA, 1991).

If the enzymes of the present invention are secreted into the nutrient medium, they may be recovered directly from the medium. If they are not secreted, they may be recovered from cell lysates. The enzymes of the present invention may be recovered from the culture medium by conventional procedures including separating the host cells from the medium by centrifugation or filtration, precipitating the proteinaceous components of the supernatant or filtrate by means of a salt, e.g. ammonium sulphate, purification by a variety of chromatographic procedures, e.g. ion exchange chromatography, gel filtration chromatography, affinity chromatography, or the like, dependent on the enzyme in question.

The enzymes of the invention may be detected using methods known in the art that are specific for these proteins. These detection methods include use of specific antibodies, formation of a product, or disappearance of a substrate. For example, an enzyme assay may be used to determine the activity of the molecule. Procedures for determining various kinds of activity are known in the art.

The enzymes of the present invention may be purified by a variety of procedures known in the art including, but not limited to, chromatography (e.g., ion exchange, affinity, hydrophobic, chromatofocusing, and size exclusion), electrophoretic procedures (e.g., preparative isoelectric focusing (IEF), differential solubility (e.g., ammonium sulfate precipitation), or extraction (see, e.g., Protein Purification, J-C Janson and Lars Ryden, editors, VCH Publishers, New York, 1989).

When an expression vector comprising a DNA sequence encoding an enzyme of the present invention is transformed/transfected into a heterologous host cell it is possible to enable heterologous recombinant production of the enzyme. An advantage of using a heterologous host cell is that it is possible to make a highly purified enzyme composition, characterized in being free from homologous impurities, which are often present when a protein or peptide is expressed in a homologous host cell. In this context homologous impurities mean any impurity (e.g. other polypeptides than the enzyme of the invention) which originates from the homologous cell where the enzyme of the invention is originally obtained from.

DETERGENT APPLICATIONS

The enzyme of the invention may be added to and thus become a component of a detergent composition.

The detergent composition of the invention may for example be formulated as a hand or machine laundry detergent composition including a laundry additive composition suitable for pre-treatment of stained fabrics and a rinse added fabric softener composition, or be formulated as a detergent composition for use in general household hard surface cleaning operations, or be formulated for hand or machine dishwashing operations.

In a specific aspect, the invention provides a detergent additive comprising the enzyme of the invention. The detergent additive as well as the detergent composition may comprise one or more other enzymes such as a protease, a lipase, a cutinase, an amylase, a carbohydrase, a cellulase, a pectinase, a mannanase, an arabinase, a galactanase, a xylanase, an oxidase, e.g., a laccase, and/or a peroxidase.

In general the properties of the chosen enzyme(s) should be compatible with the selected detergent, (i.e. pH-optimum, compatibility with other enzymatic and non-enzymatic ingredients, etc.), and the enzyme(s) should be present in effective amounts.

Proteases:

Suitable proteases include those of animal, vegetable or microbial origin. Microbial origin is preferred. Chemically modified or protein engineered mutants are included. The protease may be a serine protease or a metallo protease, preferably an alkaline microbial protease or a trypsin-like protease. Examples of alkaline proteases are subtilisins, especially those derived from *Bacillus*, e.g., subtilisin Novo, subtilisin

Carlsberg, subtilisin 309, subtilisin 147 and subtilisin 168 (described in WO 89/06279). Examples of trypsin-like proteases are trypsin (e.g. of porcine or bovine origin) and the *Fusarium* protease described in WO 89/06270 and WO 94/25583.

Examples of useful proteases are the variants described in WO 92/19729, WO 98/20115, WO 98/20116, and WO 98/34946, especially the variants with substitutions in one or more of the following positions: 27, 36, 57, 68, 76, 87, 97, 101, 104, 106, 120, 123, 167, 170, 194, 206, 218, 222, 224, 235, 245, 252 and 274.

Preferred commercially available protease enzymes include Alcalase™, Savinase™, Primase™, Duralase™, Esperase™, Coronase™ and Kannase™ (Novozymes A/S), Maxatase™, Maxacal™, Maxapem™, Properase™, Purafect™, Purafect OxP™, FN2™, and FN3™ (Genencor International Inc.).

Lipases:

Suitable lipases include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Examples of useful lipases include lipases from *Humicola* (synonym *Thermomyces*), e.g. from *H. lanuginosa* (*T. lanuginosus*) as described in EP 258 068 and EP 305 216 or from *H. insolens* as described in WO 96/13580, a *Pseudomonas* lipase, e.g. from *P. alcaligenes* or *P. pseudoalcaligenes* (EP 218 272), *P. cepacia* (EP 331 376), *P. stutzeri* (GB 1,372,034), *P. fluorescens*, *Pseudomonas sp.* strain SD 705 (WO 95/06720 and WO 96/27002), *P. wisconsinensis* (WO 96/12012), a *Bacillus* lipase, e.g. from *B. subtilis* (Dartois et al. (1993), Biochemica et Biophysica Acta, 1131, 253-360), *B. stearothermophilus* (JP 64/744992) or *B. pumilus* (WO 91/16422).

Other examples are lipase variants such as those described in WO 92/05249, WO 94/01541, EP 407 225, EP 260 105, WO 95/35381, WO 96/00292, WO 95/30744, WO 94/25578, WO 95/14783, WO 95/22615, WO 97/04079 and WO 97/07202.

Preferred commecially available lipase enzymes include Lipolase™, Lipolase Ultra™ and Lipex™ (Novozymes A/S).

Amylases:

Suitable amylases (α and/or β) include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Amylases include, for example, α-amylases obtained from *Bacillus*, e.g. a special strain of *B. licheniformis*, described in more detail in GB 1,296,839.

Examples of useful amylases are the variants described in WO 94/02597, WO 94/18314, WO 96/23873, and WO 97/43424, especially the variants with substitutions in one or more of the following positions: 15, 23, 105, 106, 124, 128, 133, 154, 156, 181, 188, 190, 197, 202, 208, 209, 243, 264, 304, 305, 391, 408, and 444.

Commercially available amylases are Duramyl™, Termamyl™, Stainzyme™ , Fungamyl™ and BAN™ (Novozymes A/S), Rapidase™ and Purastar™ (from Genencor International Inc.).

Cellulases:

Suitable cellulases include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Suitable cellulases include cellulases from the genera *Bacillus, Pseudomonas, Humicola, Fusarium, Thielavia, Acremonium,* e.g. the fungal cellulases produced from *Humicola insolens, Myceliophthora thermophila* and *Fusarium oxysporum* disclosed in US 4,435,307, US 5,648,263, US 5,691,178, US 5,776,757 and WO 89/09259.

Especially suitable cellulases are the alkaline or neutral cellulases having colour care benefits. Examples of such cellulases are cellulases described in EP 0 495 257, EP 0 531 372, WO 96/11262, WO 96/29397, WO 98/08940. Other examples are cellulase variants such as those described in WO 94/07998, EP 0 531 315, US 5,457,046, US 5,686,593, US 5,763,254, WO 95/24471, WO 98/12307 and PCT/DK98/00299.

Commercially available cellulases include Renozyme™, Celluzyme™, and Carezyme™ (Novozymes A/S), Clazinase™, and Puradax HA™ (Genencor International Inc.), and KAC-500(B)™ (Kao Corporation).

Peroxidases/Oxidases:

Suitable peroxidases/oxidases include those of plant, bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Examples of useful peroxidases include peroxidases from *Coprinus*, e.g. from *C. cinereus*, and variants thereof as those described in WO 93/24618, WO 95/10602, and WO 98/15257.

Commercially available peroxidases include Guardzyme™ (Novozymes A/S).

The detergent enzyme(s) may be included in a detergent composition by adding separate additives containing one or more enzymes, or by adding a combined additive comprising all of these enzymes. A detergent additive of the invention, i.e. a separate additive or a combined additive, can be formulated e.g. as a granulate, a liquid, a

slurry, etc. Preferred detergent additive formulations are granulates, in particular non-dusting granulates, liquids, in particular stabilized liquids, or slurries.

Non-dusting granulates may be produced, e.g., as disclosed in US 4,106,991 and 4,661,452 and may optionally be coated by methods known in the art. Examples of waxy coating materials are poly(ethylene oxide) products (polyethylene glycol, PEG) with mean molar weights of 1000 to 20000; ethoxylated nonylphenols having from 16 to 50 ethylene oxide units; ethoxylated fatty alcohols in which the alcohol contains from 12 to 20 carbon atoms and in which there are 15 to 80 ethylene oxide units; fatty alcohols; fatty acids; and mono- and di- and triglycerides of fatty acids. Examples of film-forming coating materials suitable for application by fluid bed techniques are given in GB 1483591. Liquid enzyme preparations may, for instance, be stabilized by adding a polyol such as propylene glycol, a sugar or sugar alcohol, lactic acid or boric acid according to established methods. Protected enzymes may be prepared according to the method disclosed in EP 238,216.

The detergent composition of the invention may be in any convenient form, e.g., a bar, a tablet, a powder, a granule, a paste or a liquid. A liquid detergent may be aqueous, typically containing up to 70 % water and 0-30 % organic solvent, or non-aqueous.

The detergent composition comprises one or more surfactants, which may be non-ionic including semi-polar and/or anionic and/or cationic and/or zwitterionic. The surfactants are typically present at a level of from 0.1% to 60% by weight.

When included therein the detergent will usually contain from about 1% to about 40% of an anionic surfactant such as linear alkylbenzenesulfonate, alpha-olefinsulfonate, alkyl sulfate (fatty alcohol sulfate), alcohol ethoxysulfate, secondary alkanesulfonate, alpha-sulfo fatty acid methyl ester, alkyl- or alkenylsuccinic acid or soap.

When included therein the detergent will usually contain from about 0.2% to about 40% of a non-ionic surfactant such as alcohol ethoxylate, nonylphenol ethoxylate, alkylpolyglycoside, alkyldimethylamineoxide, ethoxylated fatty acid monoethanolamide, fatty acid monoethanolamide, polyhydroxy alkyl fatty acid amide, or N-acyl N-alkyl derivatives of glucosamine ("glucamides").

The detergent may contain 0-65 % of a detergent builder or complexing agent such as zeolite, diphosphate, triphosphate, phosphonate, carbonate, citrate, nitrilotriacetic acid, ethylenediaminetetraacetic acid, diethylenetriaminepentaacetic acid, alkyl- or alkenylsuccinic acid, soluble silicates or layered silicates (e.g. SKS-6 from Hoechst).

The detergent may comprise one or more polymers. Examples are carboxy-

methylcellulose, poly(vinylpyrrolidone), poly (ethylene glycol), poly(vinyl alcohol), poly(vinylpyridine-N-oxide), poly(vinylimidazole), polycarboxylates such as polyacrylates, maleic/acrylic acid copolymers and lauryl methacrylate/acrylic acid copolymers.

The detergent may contain a bleaching system which may comprise a H_2O_2 source such as perborate or percarbonate which may be combined with a peracid-forming bleach activator such as tetraacetylethylenediamine or nonanoyloxybenzenesulfonate. Alternatively, the bleaching system may comprise peroxyacids of e.g. the amide, imide, or sulfone type.

The enzyme(s) of the detergent composition of the invention may be stabilized using conventional stabilizing agents, e.g., a polyol such as propylene glycol or glycerol, a sugar or sugar alcohol, lactic acid, boric acid, or a boric acid derivative, e.g., an aromatic borate ester, or a phenyl boronic acid derivative such as 4-formylphenyl boronic acid, and the composition may be formulated as described in e.g. WO 92/19709 and WO 92/19708.

The detergent may also contain other conventional detergent ingredients such as e.g. fabric conditioners including clays, foam boosters, suds suppressors, anti-corrosion agents, soil-suspending agents, anti-soil redeposition agents, dyes, bactericides, optical brighteners, hydrotropes, tarnish inhibitors, or perfumes.

It is at present contemplated that in the detergent compositions any enzyme, in particular the enzyme of the invention, may be added in an amount corresponding to 0.01-100 mg of enzyme protein per litre of wash liquor, preferably 0.05-5 mg of enzyme protein per litre of wash liquor, in particular 0.1-1 mg of enzyme protein per litre of wash liquor.

The enzyme of the invention may additionally be incorporated in the detergent formulations disclosed in WO 97/07202 which is hereby incorporated as reference.

FOOD PROCESSING APPLICATIONS

The RP-II protease variants of the present invention may also be used in the processing of food, especially in the field of diary products, such as milk, cream and cheese, but also in the processing of meat and vegetables.

FEED PROCESSING APPLICATION

The RP-II protease variants of the present invention may also be used in the processing of feed for cattle, poultry, and pigs and especially for pet food.

TREATMENT OF HIDES

The RP-II protease variants of the invention may also be used for the treatment of hides.

DECONTAMINATION OF POSSIBLY INFESTED MATERIALS

The RP-II protease variants of the invention may also be used in processes for decontaminating instruments, surfaces, and other materials in hospitals, clinics, and meat processing plants, etc. in order to decompose prions or other infectious agents.

MATERIALS AND METHODS

Strains:

B. subtilis DN1885: Disclosed in WO 01/16285

Plasmids:

pNM1003: Disclosed in WO 01/16285
pSX222: Disclosed in WO 96/34946
pNM1008: See Example 2

Method for producing a protease variant

The present invention provides a method of producing an isolated enzyme according to the invention, wherein a suitable host cell, which has been transformed with a DNA sequence encoding the enzyme, is cultured under conditions permitting the production of the enzyme, and the resulting enzyme is recovered from the culture.

When an expression vector comprising a DNA sequence encoding the enzyme is transformed into a heterologous host cell it is possible to enable heterologous recombinant production of the enzyme of the invention. Thereby it is possible to make a highly purified RP-II protease composition, characterized in being free from homologous impurities.

The medium used to culture the transformed host cells may be any conventional medium suitable for growing the host cells in question. The expressed RP-II protease

may conveniently be secreted into the culture medium and may be recovered therefrom by well-known procedures including separating the cells from the medium by centrifugation or filtration, precipitating proteinaceous components of the medium by means of a salt such as ammonium sulfate, followed by chromatographic procedures such as ion exchange chromatography, affinity chromatography, or the like.

Proteolytic Activity

Enzyme activity can be measured using the PNA assay using succinyl-alanine-alanine-proline-glutamicacid-paranitroaniline as a substrate. The principle of the PNA assay is described in the Journal of American Oil Chemists Society, Rothgeb, T.M., Goodlander, B.D., Garrison, P.H., and Smith, L.A., (1988).

Textiles

Standard textile pieces are obtained from EMPA St. Gallen, Lerchfeldstrasse 5, CH-9014 St. Gallen, Switzerland. Especially type EMPA 116 (cotton textile stained with blood, milk and ink) and EMPA 117 (polyester/cotton textile stained with blood, milk and ink). The textile can be cut into a smaller textile piece of 5x3 cm or 13x3 cm

Other relevant protease stain may be used as well, e.g. C-03, C-05, C-10 from CFT, Center For Testmaterials, Vlaardingen, Netherlands

Wash Conditions

Region	Latin America	Europe	North America	Japan
Temperature	20°C	30°C	20°C	20°C
Washing time	14 min	20 min	12 min	15 min
Swatches	EMPA 117	EMPA 116	EMPA 117	EMPA 117
Water Hardness*	9 or 12°dH	15°dH	6°dH	3°dH
Detergent dosage	1.5 or 2.5 g/l	4, 6 or 8 g/l	HDL: 1.5 g/l	0.5 or 0.7
Washing pH	As is, or adjusted to 8, 9, 10	As is, or adjusted to 8, 9, 10	As is, or adjusted to 8, 9, 10	As is, or adjusted to 8, 9, 10

* °dH: adjusted by adding $CaCl_2*2H_2O$; $MgCl_2*6H_2O$; $NaHCO_3$ (Ratio Ca^{2+}:Mg^{2+}:HCO^{3-} = 2:1:6) to milli-Q water.

Detergents

The enzymes of the invention may be tested in the detergent formulations disclosed in WO 97/07202 or in detergents formulations purchased from wfk testgewebe GmbH or similar supplier

List of test detergents from wfk testgewebe

- IEC 60456 Type A* Base Detergent
- IEC 60456 Type B Base Detergent
- IEC 60456 Type C Detergent
- ECE Reference Detergent with Phosphate (1977)
- ECE Reference Detergent without Phosphate (1998)
- AHAM Standard Detergent
- EU ECOLABEL (detergents) Light Duty Detergent
- EU ECOLABEL (detergents) PVP

However, also one of the following commercial detergents may be used in the wash assay, e.g.

- Omo Multi Acao HDP, Unilever, Brazil
- Tide HDL, P&G, US
- Wisk HDL, Unilever, US
- TOP HDP, Lion, Japan
- Attack HDP, Kao, Japan
- Ariel Regular HDP, P&G, Europe
- Ariel Compact HDPC, P&G, Europe
- Persil Megaperls, Henkel, Germany
- Persil, Unilever, UK

Furthermore, a brand extension or color / compact version for the above specified detergent could be used as well

If the detergent contains enzymes, the detergent should be in-activated before use in order to eliminate the enzyme activity already present in the detergent. This is done by heating a detergent stock solution to 85°C in 5 minutes in a micro wave oven. The concentration of

the detergent stock solution in the micro wave oven is between 4 – 20 g/l

EXAMPLE 1

Modelling RP-II Proteases from the 3D Structure of BLC

The overall homology of *Bacillus licheniformis* protease BCL to other RP-II proteases is high. The similarity between the different RP-II proteases is provided in Table 1. Using the sequence alignment of Fig. 2 a model of the JA96 protease can be build using a suitable modelling tool like the Accellrys software Homology , or Modeller (also from Accellrys), or other software like Nest. These programs provide results as a first rough model, with some optimization in the Modeller and Nest programs.

The first rough model provides a close structural homology between the model of JA96 protease and the 3D structure of the BCL as there are no overlapping side chains in the model structure. To optimize the structure the protein can *in silico* be soaked in a box of water and subjected to energy minimization and further molecular dynamics simulations using e.g. the CHARMm™ software from Accelrys. The *in silico* soaking in water can conveniently be done by adding water in the Insight II program (from Accelrys) with a box size of 75*75*75Å^3. The energy minimization can be done using settings of 300 Steepest descent (SD) and further 600 Conjugated gradients (CJ). The molecular dynamics simulations can conveniently be done using 1.2 ns run using the Verlet algorithm at 300K and standard parameters (see CHARMm manual). Other RP-II protease 3D models may be built in an analogous way.

EXAMPLE 2

Construction of Library of RP-II Protease Variants

Construction and Expression of BLC

A *B. subtilis – E. coli* shuttle vector, pNM1003, suited to a gene coding for RP-II protease BLC and its mutants was constructed. It is derived from the B. subtilis expression vector pSX222 (Described in WO 96/34946) as described in WO 01/16285. To facilitate cloning pNM1008 was constructed introducing a kpnI restriction site downstream the HindIII site to facilitate the cloning of fragments inside the vector. For transformation in Bacillus pNM1008 was restricted with HindIII and a 4350 bp DNA fragment was isolated and ligated. The ligation mixture was used to transform competent *B. subtilis* DN1885, selecting for protease activity, as described in WO 01/16285.

Site-directed mutagenesis

BLC site-directed variants of the invention comprising specific substitutions, insertions or deletions in the molecule are made by traditional cloning of PCR fragments (Sambrook et. al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor) produced by oligonucleotides containing the desired modification. As template pNM1008 is used. In a first PCR using a mutational primer (anti-sense) with a suitable opposite sense primer (e. g.. 5´-CTGTGCCCTTTAACCGCACAGC (SEQ ID No. 17)), downstream of the MluI site is used. The resulting DNA fragment is used as a sense primer in a second PCR together with a suitable anti-sense primer (e. g. 5´-GCATAAGCTTTTACAGGTACCGGC (SEQ ID No. 18)) upstream from the KpnI digestion site. This resulting PCR product is digested with KpnI and MluI and ligated in pNM1008 digested with the respective enzymes.

The ligation reaction is transformed into E. coli by well-known techniques and 5 randomly chosen colonies are sequenced to confirm the designed mutations.

In order to express a BLC variant of the invention, the pNM1008 derived plasmid comprising the variant is digested with HindIII, ligated and transformed into a competent B. subtilis strain, selecting for protease activity.

EXAMPLE 3

Purification of Enzymes and Variants:

This procedure relates to purification of 2 liter scale fermentation for the production of the RP-II proteases of the invention in a *Bacillus* host cell.

Approximately 1.6 liters of fermentation broth are centrifuged at 5000 rpm for 35 minutes in 1 liter beakers. The supernatants are adjusted to pH 7 using 10% acetic acid and filtered through a Seitz Supra S100 filter plate.

At room temperature, the filtrate is applied to a 100 ml Bacitracin affinity column equilibrated with 0.01M dimethylglutaric acid, 0.1 M boric acid and 0.002 M calcium chloride adjusted to pH 7 with sodium hydroxide (Buffer A). After washing the column with Buffer A to remove unbound protein, the protease is eluted from the Bacitracin column using Buffer A supplemented with 25% 2-propanol and 1 M sodium chloride.

The fractions with protease activity from the Bacitracin purification step are

combined and applied to a 750 ml Sephadex G25 column (5 cm dia.) equilibrated with Buffer A.

Fractions with proteolytic activity from the Sephadex G25 column are combined and the pH was adjusted to pH 6 with 10% acetic acid and applied to a 150 ml CM Sepharose CL 6B cation exchange column (5 cm dia.) equilibrated with a buffer containing 0.01 M dimethylglutaric acid, 0.1 M boric acid, and 0.002 M calcium chloride adjusted to pH 6 with sodium hydroxide.

The protease is eluted using a linear gradient of 0-0.2 M sodium chloride in 2 liters of the same buffer.

Finally, the protease containing fractions from the CM Sepharose column are combined and filtered through a 0.2μ filter.

By using the techniques of Example 2 for the construction of variants and fermentation, and the above isolation procedure the following RP-II proteases and variants thereof may be produced and isolated:

EXAMPLE 4

Wash Performance of Detergent Compositions Comprising Modified Enzymes

AMSA

The enzyme variants of the present application is tested using the Automatic Mechanical Stress Assay (AMSA). With the AMSA test the wash performance of a large quantity of small volume enzyme-detergent solutions can be examined. The AMSA plate has a number of slots for test solutions and a lid firmly squeezing the textile swatch to be washed against all the slot openings. During the washing time, the plate, test solutions, textile and lid are vigorously shaken to bring the test solution in contact with the textile and apply mechanical stress. For further description see WO 02/42740 especially the paragraph "Special method embodiments" at page 23-24.

The assay is conducted under the experimental conditions specified below. In respect of the detergent used, all the detergents listed above under "Materials and

Methods" may be used:

Detergent base	Example: Omo Acao
Detergent dosage	Example: 1.5 g/l
Test solution volume	160 micro l
pH	Example: As is
Wash time	Example: 14 minutes
Temperature	Example: 20°C
Water hardness	Example: 9°dH
Enzyme concentration in test solution	5 nM, 10 nM and 30 nM
Test material	Example: EMPA 117

After washing the textile pieces is flushed in tap water and air-dried.

The performance of the enzyme variant is measured as the brightness of the colour of the textile samples washed with that specific enzyme variant. Brightness can also be expressed as the intensity of the light reflected from the textile sample when luminated with white light. When the textile is stained the intensity of the reflected light is lower, than that of a clean textile. Therefore the intensity of the reflected light can be used to measure wash performance of an enzyme variant.

Colour measurements are made with a professional flatbed scanner (*PFU DL2400pro*), which is used to capture an image of the washed textile samples. The scans are made with a resolution of 200 dpi and with an output colour dept of 24 bits. In order to get accurate results, the scanner is frequently calibrated with a *Kodak reflective IT8 target*.

To extract a value for the light intensity from the scanned images, a special designed software application is used (*Novozymes Color Vector Analyzer*). The program retrieves the 24 bit pixel values from the image and converts them into values for red, green and blue (RGB). The intensity value (Int) is calculated by adding the RGB values together as vectors and then taking the length of the resulting vector:

$$Int = \sqrt{r^2 + g^2 + b^2}$$

The wash performance (P) of the variants is calculated in accordance with the below formula:

$$P = Int(v) - Int(r)$$

where

Int(v) is the light intensity value of textile surface washed with enzyme variant and

Int(r) is the light intensity value of textile surface washed with the reference enzyme BLC.

A performance score is given as the result of the miniwash in accordance with the definition:

Performance Scores (S) are summing up the performances (P) of the tested enzyme variants as:

S = 2 which indicates that the variant performs better than the reference at all three concentrations (5, 10 and 30 nM) and

S = 1 which indicates that the variant performs better than the reference at one or two concentrations.

A variant is considered to exhibit improved wash performance, if it performs better than the reference in at least one detergent composition.

Mini wash assay

The millilitre scale wash performance assay is conducted under the following conditions:

Detergent base	Example: Omo Acao detergent powder
Detergent dose	Example: 1.5 g/l
pH	Example: "as is" in the current detergent solution and is not adjusted.
Wash time	Example: 14 min.
Temperature	Example: 20°C
Water hardness	Example: 9°dH, adjusted by adding $CaCl_2*2H_2O$; $MgCl_2*6H_2O$; $NaHCO_3$ (Ca^{2+}:Mg^{2+}:HCO^{3-} = 2:1:6) to milli-Q water.
Enzymes	Variants of BLC. BLC as reference enzyme
Enzyme conc.	5 nM, 10 nM, 30 nM
Test system	125 ml glass beakers. Textile dipped in test solution. Continuously lifted up and down into the detergent solution, 50 times per minute (up-time 0.4 sec, down-time 0.4 sec, lift time 0.2 sec)
Test solution volume	50 ml
Test material	Example: EMPA 117 textile swatches (13x5 cm)

After washing the textile piece is flushed in tap water and air-dried and the remission from the test material is measured at 460 nm using a Zeiss MCS 521 VIS spectrophotometer. The measurements are done according to the manufacturer's protocol.

A performance score is given as the result of the miniwash in accordance with the definition:

Performance Scores (S) are summing up the performances (P) of the tested enzyme variants as:

S = 2 which indicates that the variant performs better than the reference at all three concentrations (5, 10 and 30 nM) and

S = 1 which indicates that the variant performs better than the reference at one or two concentrations.

A Performance Score higher than 1 indicates better wash performance.

A variant is considered to exhibit improved wash performance, if it performs better than the reference in at least one detergent composition.

The following RP-II variants were constructed as indicated in Example 2 to be purified in accordance with Example 3 and tested as indicated above:

Ion-binding modification:

D7E; D7Q; H144R; D161R; D161K;

H144Q+D161R

Mobility modification:

G30A; G91A

Cys-bridge formation:

S145C+T128C

Surface charge modification:

D7N,S,T; Y17R,K,H; Y95R,K,H; T109R,K,H; Q143R,K,H; Q174R,K,H; E209Q,N; N216R,K,H

Proline stability:

T60P; S221P; G193P; V194P

EXAMPLE 5

Storage Stability of Modified Enzymes

The storage stability of the variants of the invention is determined by measuring the "residual activity" of the parent and the variants at regular time intervals. The storage stability is often expressed as the half-life, $T_{1/2}$, the time lapsed till the activity is half the initial value.

Residual activity = (Activity at t=i)/((Activity at t=0)×100)%

The Proteolytic activity is measured as described above(PNA assay).

EXAMPLE 6

Thermostability of Modified Enzymes

The thermostability of the protease variant s of the invention is determine by Dif-

ferential Scanning Calorimetry (DSC) typically with a heating rate of 0.5°C per minute in a solution containg about 2mg/ml variant.

EXAMPLE 7

Autoproteolytic Stability Of Modified Enzymes

Comparative fermentation experiment

The RP-II variants of the invention are in a fermentation experiment compared to the parent RP-II protease.

Both the variants and the parent are cloned in a pNM1008 expression vector background and fermented in a suitable medium.

After 5 days fermentation 1.5 ml of the fermentation medium is centrifuged and the supernatant used to measure the Proteolytic activity (KPNU) as described above.

The variants providing an increased proteolytic activity in comparison to the activity of the parent are considered to posses an improved autoproteolytic stability relative to the parent.

EXAMPLE 8

Oxidation Stability of Modified Enzymes

The variants are tested for their oxidation stability in 0.01 M peracetic acid after 20 minutes at 50°C and pH 7. The parent protease is used as reference.

The results are presented by the residual proteolytic activity in the heat treated samples relative to samples untreated by oxidant or heat.

Appendix 1

```
ATOM   3359  N   SER B   1      -2.987  12.370  17.565  1.00  7.82           N
ATOM   3361  CA  SER B   1      -2.255  12.820  16.353  1.00  7.97           C
ATOM   3363  CB  SER B   1      -3.233  12.933  15.188  1.00  8.69           C
ATOM   3366  OG  SER B   1      -3.995  11.748  15.028  1.00  9.01           O
ATOM   3368  C   SER B   1      -1.637  14.171  16.602  1.00  8.14           C
ATOM   3369  O   SER B   1      -2.098  14.938  17.439  1.00  8.05           O
ATOM   3372  N   VAL B   2      -0.592  14.472  15.848  1.00  8.60           N
ATOM   3374  CA  VAL B   2      -0.039  15.812  15.824  1.00 10.11           C
ATOM   3376  CB  VAL B   2       1.432  15.811  15.404  1.00 11.81           C
ATOM   3378  CG1 VAL B   2       1.949  17.239  15.233  1.00 13.46           C
ATOM   3382  CG2 VAL B   2       2.255  15.065  16.421  1.00 14.12           C
ATOM   3386  C   VAL B   2      -0.867  16.605  14.830  1.00 10.56           C
ATOM   3387  O   VAL B   2      -0.928  16.250  13.660  1.00 12.81           O
ATOM   3388  N   ILE B   3      -1.524  17.640  15.331  1.00  9.91           N
ATOM   3390  CA  ILE B   3      -2.409  18.487  14.537  1.00 10.49           C
ATOM   3392  CB  ILE B   3      -3.747  18.700  15.279  1.00 10.68           C
ATOM   3394  CG1 ILE B   3      -4.452  17.348  15.457  1.00 10.36           C
ATOM   3397  CD1 ILE B   3      -5.671  17.398  16.350  1.00 11.17           C
ATOM   3401  CG2 ILE B   3      -4.638  19.704  14.531  1.00 13.34           C
ATOM   3405  C   ILE B   3      -1.683  19.796  14.299  1.00 10.96           C
ATOM   3406  O   ILE B   3      -1.332  20.502  15.234  1.00 10.91           O
ATOM   3407  N   GLY B   4      -1.433  20.141  13.043  1.00 12.22           N
ATOM   3409  CA  GLY B   4      -0.702  21.359  12.748  1.00 12.69           C
ATOM   3412  C   GLY B   4       0.685  21.285  13.344  1.00 12.61           C
ATOM   3413  O   GLY B   4       1.324  20.239  13.303  1.00 13.40           O
ATOM   3414  N   SER B   5       1.162  22.383  13.913  1.00 11.93           N
ATOM   3416  CA  SER B   5       2.466  22.358  14.557  1.00 11.64           C
ATOM   3418  CB  SER B   5       2.900  23.757  14.975  1.00 11.92           C
ATOM   3421  OG  SER B   5       2.011  24.329  15.906  1.00 13.28           O
ATOM   3423  C   SER B   5       2.438  21.451  15.770  1.00 11.22           C
ATOM   3424  O   SER B   5       1.437  21.366  16.462  1.00 11.19           O
ATOM   3425  N   ASP B   6       3.551  20.779  16.028  1.00 10.41           N
ATOM   3427  CA  ASP B   6       3.704  19.951  17.230  1.00 10.02           C
ATOM   3429  CB  ASP B   6       4.700  18.839  16.981  1.00 10.75           C
ATOM   3432  CG  ASP B   6       4.838  17.886  18.144  1.00 10.38           C
ATOM   3433  OD1 ASP B   6       4.132  18.013  19.178  1.00 10.80           O
ATOM   3434  OD2 ASP B   6       5.685  16.961  18.055  1.00 11.46           O
ATOM   3435  C   ASP B   6       4.185  20.807  18.373  1.00  9.61           C
ATOM   3436  O   ASP B   6       5.353  21.229  18.410  1.00 11.09           O
ATOM   3437  N   ASP B   7       3.290  21.057  19.312  1.00  8.85           N
ATOM   3439  CA  ASP B   7       3.582  21.969  20.387  1.00  8.21           C
ATOM   3441  CB  ASP B   7       2.453  23.010  20.550  1.00  9.26           C
ATOM   3444  CG  ASP B   7       2.334  23.975  19.386  1.00 10.17           C
ATOM   3445  OD1 ASP B   7       3.147  23.902  18.444  1.00 11.15           O
ATOM   3446  OD2 ASP B   7       1.377  24.778  19.332  1.00 10.99           O
ATOM   3447  C   ASP B   7       3.856  21.237  21.712  1.00  8.24           C
ATOM   3448  O   ASP B   7       3.978  21.870  22.753  1.00  8.50           O
ATOM   3449  N   ARG B   8       4.016  19.918  21.677  1.00  7.90           N
ATOM   3451  CA  ARG B   8       4.429  19.187  22.872  1.00  7.81           C
ATOM   3453  CB  ARG B   8       4.444  17.681  22.634  1.00  7.75           C
ATOM   3456  CG  ARG B   8       3.068  17.077  22.470  1.00  7.65           C
ATOM   3459  CD  ARG B   8       3.090  15.631  22.015  1.00  7.89           C
ATOM   3462  NE  ARG B   8       3.673  15.554  20.679  1.00  8.24           N
ATOM   3464  CZ  ARG B   8       4.023  14.422  20.073  1.00  8.49           C
ATOM   3465  NH1 ARG B   8       3.781  13.244  20.628  1.00  8.61           N
ATOM   3468  NH2 ARG B   8       4.622  14.472  18.909  1.00  9.63           N
ATOM   3471  C   ARG B   8       5.812  19.628  23.321  1.00  8.24           C
ATOM   3472  O   ARG B   8       6.684  19.907  22.505  1.00  9.34           O
ATOM   3473  N   THR B   9       6.007  19.640  24.632  1.00  8.26           N
ATOM   3475  CA  THR B   9       7.315  19.897  25.226  1.00  8.75           C
ATOM   3477  CB  THR B   9       7.368  21.243  25.939  1.00  9.87           C
ATOM   3479  OG1 THR B   9       6.296  21.350  26.880  1.00 10.91           O
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ATOM   3481  CG2 THR B   9       7.191  22.375  24.936  1.00 11.78           C
ATOM   3485  C   THR B   9       7.660  18.787  26.199  1.00  8.34           C
ATOM   3486  O   THR B   9       6.793  18.176  26.835  1.00  8.22           O
ATOM   3487  N   ARG B  10       8.954  18.535  26.340  1.00  8.65           N
ATOM   3489  CA  ARG B  10       9.413  17.459  27.194  1.00  8.98           C
ATOM   3491  CB  ARG B  10      10.873  17.096  26.927  1.00 10.45           C
ATOM   3494  CG  ARG B  10      11.309  15.787  27.587  1.00 11.25           C
ATOM   3497  CD  ARG B  10      12.701  15.396  27.212  1.00 12.23           C
ATOM   3500  NE  ARG B  10      13.213  14.299  28.025  1.00 12.62           N
ATOM   3502  CZ  ARG B  10      14.465  13.868  27.967  1.00 14.40           C
ATOM   3503  NH1 ARG B  10      15.328  14.413  27.114  1.00 16.93           N
ATOM   3506  NH2 ARG B  10      14.855  12.884  28.743  1.00 14.13           N
ATOM   3509  C   ARG B  10       9.237  17.885  28.642  1.00  8.65           C
ATOM   3510  O   ARG B  10       9.534  19.027  29.025  1.00  9.59           O
ATOM   3511  N   VAL B  11       8.771  16.952  29.453  1.00  8.69           N
ATOM   3513  CA  VAL B  11       8.751  17.118  30.893  1.00  9.52           C
ATOM   3515  CB  VAL B  11       7.810  16.080  31.532  1.00  9.21           C
ATOM   3517  CG1 VAL B  11       7.862  16.145  33.047  1.00 10.41           C
ATOM   3521  CG2 VAL B  11       6.381  16.257  31.015  1.00  9.54           C
ATOM   3525  C   VAL B  11      10.207  16.954  31.390  1.00 10.62           C
ATOM   3526  O   VAL B  11      10.777  15.869  31.301  1.00 12.34           O
ATOM   3527  N   THR B  12      10.795  18.048  31.884  1.00 12.38           N
ATOM   3529  CA  THR B  12      12.217  18.113  32.253  1.00 13.55           C
ATOM   3531  CB  THR B  12      12.790  19.543  32.093  1.00 14.37           C
ATOM   3533  OG1 THR B  12      12.035  20.449  32.902  1.00 17.60           O
ATOM   3535  CG2 THR B  12      12.611  20.030  30.671  1.00 16.03           C
ATOM   3539  C   THR B  12      12.507  17.657  33.666  1.00 13.34           C
ATOM   3540  O   THR B  12      13.669  17.515  34.032  1.00 14.60           O
ATOM   3541  N   ASN B  13      11.472  17.465  34.469  1.00 12.04           N
ATOM   3543  CA  ASN B  13      11.646  16.901  35.800  1.00 11.12           C
ATOM   3545  CB  ASN B  13      11.713  17.962  36.894  1.00 11.74           C
ATOM   3548  CG  ASN B  13      11.935  17.344  38.252  1.00 12.29           C
ATOM   3549  OD1 ASN B  13      12.166  16.141  38.356  1.00 12.18           O
ATOM   3550  ND2 ASN B  13      11.868  18.153  39.302  1.00 15.45           N
ATOM   3553  C   ASN B  13      10.502  15.940  36.074  1.00 10.21           C
ATOM   3554  O   ASN B  13       9.450  16.321  36.578  1.00 10.60           O
ATOM   3555  N   THR B  14      10.714  14.678  35.743  1.00  9.43           N
ATOM   3557  CA  THR B  14       9.671  13.680  35.934  1.00  9.11           C
ATOM   3559  CB  THR B  14       9.887  12.455  35.046  1.00  9.24           C
ATOM   3561  OG1 THR B  14      11.122  11.827  35.409  1.00  9.63           O
ATOM   3563  CG2 THR B  14       9.958  12.808  33.561  1.00 10.29           C
ATOM   3567  C   THR B  14       9.556  13.227  37.385  1.00  9.62           C
ATOM   3568  O   THR B  14       8.730  12.361  37.672  1.00 10.68           O
ATOM   3569  N   THR B  15      10.357  13.804  38.295  1.00 10.09           N
ATOM   3571  CA  THR B  15      10.147  13.593  39.725  1.00 10.57           C
ATOM   3573  CB  THR B  15      11.456  13.495  40.553  1.00 11.89           C
ATOM   3575  OG1 THR B  15      12.124  14.763  40.616  1.00 12.96           O
ATOM   3577  CG2 THR B  15      12.432  12.491  39.954  1.00 12.96           C
ATOM   3581  C   THR B  15       9.244  14.638  40.367  1.00 10.41           C
ATOM   3582  O   THR B  15       8.911  14.514  41.540  1.00 12.03           O
ATOM   3583  N   ALA B  16       8.832  15.656  39.622  1.00 10.32           N
ATOM   3585  CA  ALA B  16       7.900  16.643  40.148  1.00 10.73           C
ATOM   3587  CB  ALA B  16       7.927  17.897  39.301  1.00 11.48           C
ATOM   3591  C   ALA B  16       6.488  16.060  40.161  1.00 10.05           C
ATOM   3592  O   ALA B  16       6.059  15.433  39.198  1.00  9.80           O
ATOM   3593  N   TYR B  17       5.755  16.284  41.237  1.00 10.35           N
ATOM   3595  CA  TYR B  17       4.338  15.962  41.260  1.00 10.36           C
ATOM   3597  CB  TYR B  17       3.838  16.018  42.706  1.00 10.90           C
ATOM   3600  CG  TYR B  17       2.379  15.675  42.858  1.00 10.77           C
ATOM   3601  CD1 TYR B  17       1.436  16.674  42.985  1.00 11.41           C
ATOM   3603  CE1 TYR B  17       0.086  16.386  43.118  1.00 11.35           C
ATOM   3605  CZ  TYR B  17      -0.338  15.081  43.139  1.00 11.51           C
ATOM   3606  OH  TYR B  17      -1.690  14.831  43.268  1.00 13.22           O
ATOM   3608  CE2 TYR B  17       0.579  14.051  42.988  1.00 11.13           C
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ATOM   3610  CD2 TYR B  17       1.940  14.358  42.861  1.00 11.24           C
ATOM   3612  C   TYR B  17       3.588  16.946  40.363  1.00 10.06           C
ATOM   3613  O   TYR B  17       3.857  18.150  40.452  1.00 11.57           O
ATOM   3614  N   PRO B  18       2.609  16.510  39.557  1.00 10.05           N
ATOM   3615  CA  PRO B  18       2.080  15.145  39.436  1.00  9.55           C
ATOM   3617  CB  PRO B  18       0.606  15.412  39.151  1.00 10.69           C
ATOM   3620  CG  PRO B  18       0.646  16.604  38.275  1.00 11.31           C
ATOM   3623  CD  PRO B  18       1.772  17.460  38.810  1.00 10.99           C
ATOM   3626  C   PRO B  18       2.667  14.326  38.287  1.00  8.62           C
ATOM   3627  O   PRO B  18       2.189  13.217  38.035  1.00  8.43           O
ATOM   3628  N   TYR B  19       3.695  14.844  37.616  1.00  8.36           N
ATOM   3630  CA  TYR B  19       4.343  14.126  36.531  1.00  8.21           C
ATOM   3632  CB  TYR B  19       5.389  15.034  35.875  1.00  8.56           C
ATOM   3635  CG  TYR B  19       4.722  16.277  35.304  1.00  8.70           C
ATOM   3636  CD1 TYR B  19       4.072  16.231  34.070  1.00  8.24           C
ATOM   3638  CE1 TYR B  19       3.424  17.343  33.553  1.00  9.10           C
ATOM   3640  CZ  TYR B  19       3.374  18.496  34.286  1.00  9.96           C
ATOM   3641  OH  TYR B  19       2.725  19.608  33.802  1.00 11.01           O
ATOM   3643  CE2 TYR B  19       3.987  18.565  35.519  1.00 10.79           C
ATOM   3645  CD2 TYR B  19       4.660  17.462  36.020  1.00 10.02           C
ATOM   3647  C   TYR B  19       4.951  12.801  36.969  1.00  7.80           C
ATOM   3648  O   TYR B  19       4.984  11.860  36.180  1.00  8.04           O
ATOM   3649  N   ARG B  20       5.385  12.701  38.224  1.00  7.62           N
ATOM   3651  CA  ARG B  20       5.919  11.452  38.741  1.00  7.92           C
ATOM   3653  CB  ARG B  20       6.659  11.679  40.056  1.00  8.70           C
ATOM   3656  CG  ARG B  20       5.865  12.292  41.176  1.00  9.58           C
ATOM   3659  CD  ARG B  20       6.640  12.228  42.469  1.00 10.61           C
ATOM   3662  NE  ARG B  20       5.937  12.768  43.620  1.00 12.27           N
ATOM   3664  CZ  ARG B  20       6.343  13.830  44.332  1.00 14.55           C
ATOM   3665  NH1 ARG B  20       7.433  14.528  44.011  1.00 15.43           N
ATOM   3668  NH2 ARG B  20       5.641  14.205  45.395  1.00 15.98           N
ATOM   3671  C   ARG B  20       4.833  10.398  38.938  1.00  7.88           C
ATOM   3672  O   ARG B  20       5.142   9.210  39.062  1.00  8.74           O
ATOM   3673  N   ALA B  21       3.573  10.834  38.989  1.00  7.67           N
ATOM   3675  CA  ALA B  21       2.436   9.931  39.101  1.00  7.77           C
ATOM   3677  CB  ALA B  21       1.355  10.545  40.004  1.00  8.33           C
ATOM   3681  C   ALA B  21       1.860   9.554  37.740  1.00  7.49           C
ATOM   3682  O   ALA B  21       0.883   8.813  37.670  1.00  8.24           O
ATOM   3683  N   ILE B  22       2.451  10.077  36.668  1.00  7.07           N
ATOM   3685  CA  ILE B  22       2.180   9.629  35.315  1.00  7.15           C
ATOM   3687  CB  ILE B  22       2.239  10.805  34.320  1.00  7.19           C
ATOM   3689  CG1 ILE B  22       1.204  11.861  34.727  1.00  7.74           C
ATOM   3692  CD1 ILE B  22       1.150  13.060  33.823  1.00  7.78           C
ATOM   3696  CG2 ILE B  22       2.012  10.301  32.895  1.00  7.55           C
ATOM   3700  C   ILE B  22       3.192   8.540  35.014  1.00  7.08           C
ATOM   3701  O   ILE B  22       4.376   8.686  35.297  1.00  8.15           O
ATOM   3702  N   VAL B  23       2.708   7.426  34.477  1.00  7.33           N
ATOM   3704  CA  VAL B  23       3.505   6.221  34.384  1.00  7.49           C
ATOM   3706  CB  VAL B  23       2.933   5.092  35.284  1.00  7.65           C
ATOM   3708  CG1 VAL B  23       2.619   5.599  36.672  1.00  8.69           C
ATOM   3712  CG2 VAL B  23       1.690   4.436  34.682  1.00  8.21           C
ATOM   3716  C   VAL B  23       3.625   5.760  32.939  1.00  6.99           C
ATOM   3717  O   VAL B  23       2.710   5.912  32.130  1.00  7.44           O
ATOM   3718  N   HIS B  24       4.788   5.194  32.623  1.00  7.09           N
ATOM   3720  CA  HIS B  24       5.005   4.494  31.375  1.00  7.24           C
ATOM   3722  CB  HIS B  24       6.484   4.596  30.984  1.00  7.56           C
ATOM   3725  CG  HIS B  24       6.810   3.808  29.779  1.00  8.11           C
ATOM   3726  ND1 HIS B  24       7.112   2.467  29.831  1.00  9.52           N
ATOM   3728  CE1 HIS B  24       7.263   2.022  28.599  1.00 10.58           C
ATOM   3730  NE2 HIS B  24       7.090   3.026  27.757  1.00 11.37           N
ATOM   3732  CD2 HIS B  24       6.804   4.156  28.474  1.00 10.43           C
ATOM   3734  C   HIS B  24       4.599   3.027  31.568  1.00  7.57           C
ATOM   3735  O   HIS B  24       4.949   2.409  32.577  1.00  8.17           O
ATOM   3736  N   ILE B  25       3.848   2.485  30.615  1.00  7.37           N
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ATOM   3738  CA   ILE B  25       3.381   1.108  30.652  1.00  7.87           C
ATOM   3740  CB   ILE B  25       1.842   1.058  30.651  1.00  8.18           C
ATOM   3742  CG1  ILE B  25       1.257   1.843  31.824  1.00  9.00           C
ATOM   3745  CD1  ILE B  25      -0.242   2.093  31.705  1.00  8.99           C
ATOM   3749  CG2  ILE B  25       1.356  -0.398  30.666  1.00  9.66           C
ATOM   3753  C    ILE B  25       3.899   0.364  29.441  1.00  8.15           C
ATOM   3754  O    ILE B  25       3.755   0.843  28.315  1.00  8.94           O
ATOM   3755  N    SER B  26       4.486  -0.806  29.669  1.00  8.77           N
ATOM   3757  CA   SER B  26       4.773  -1.727  28.581  1.00  9.89           C
ATOM   3759  CB BSER B  26       6.238  -1.804  28.196  0.35 10.66           C
ATOM   3760  CB ASER B  26       6.305  -1.864  28.514  0.65 11.47           C
ATOM   3765  OG BSER B  26       6.986  -2.328  29.246  0.35 11.77           O
ATOM   3766  OG ASER B  26       6.755  -2.916  27.701  0.65 12.82           O
ATOM   3769  C    SER B  26       4.177  -3.089  28.889  1.00  9.15           C
ATOM   3770  O    SER B  26       4.245  -3.579  30.017  1.00  9.90           O
ATOM   3771  N    SER B  27       3.579  -3.695  27.878  1.00  8.91           N
ATOM   3773  CA   SER B  27       3.049  -5.042  27.993  1.00  9.24           C
ATOM   3775  CB   SER B  27       1.609  -5.020  28.523  1.00  9.75           C
ATOM   3778  OG   SER B  27       0.701  -4.659  27.498  1.00 10.07           O
ATOM   3780  C    SER B  27       3.045  -5.686  26.626  1.00  9.09           C
ATOM   3781  O    SER B  27       3.418  -5.071  25.633  1.00  9.64           O
ATOM   3782  N    SER B  28       2.555  -6.913  26.573  1.00  9.24           N
ATOM   3784  CA   SER B  28       2.448  -7.620  25.319  1.00  9.63           C
ATOM   3786  CB   SER B  28       1.950  -9.034  25.569  1.00 10.05           C
ATOM   3789  OG   SER B  28       0.663  -9.022  26.149  1.00 11.00           O
ATOM   3791  C    SER B  28       1.551  -6.906  24.309  1.00  9.09           C
ATOM   3792  O    SER B  28       1.683  -7.141  23.109  1.00 10.26           O
ATOM   3793  N    ILE B  29       0.612  -6.081  24.765  1.00  9.01           N
ATOM   3795  CA   ILE B  29      -0.230  -5.322  23.829  1.00  9.45           C
ATOM   3797  CB   ILE B  29      -1.528  -4.860  24.527  1.00  9.84           C
ATOM   3799  CG1  ILE B  29      -2.467  -6.054  24.687  1.00 10.68           C
ATOM   3802  CD1  ILE B  29      -3.749  -5.729  25.407  1.00 11.23           C
ATOM   3806  CG2  ILE B  29      -2.209  -3.738  23.755  1.00 10.93           C
ATOM   3810  C    ILE B  29       0.520  -4.165  23.182  1.00  9.75           C
ATOM   3811  O    ILE B  29       0.298  -3.856  22.009  1.00 10.61           O
ATOM   3812  N    GLY B  30       1.392  -3.519  23.936  1.00  9.50           N
ATOM   3814  CA   GLY B  30       2.104  -2.366  23.439  1.00 10.18           C
ATOM   3817  C    GLY B  30       2.498  -1.451  24.564  1.00  8.93           C
ATOM   3818  O    GLY B  30       2.432  -1.827  25.728  1.00 10.65           O
ATOM   3819  N    SER B  31       2.926  -0.258  24.195  1.00  9.21           N
ATOM   3821  CA   SER B  31       3.322   0.746  25.151  1.00  9.76           C
ATOM   3823  CB BSER B  31       4.627   1.413  24.672  0.35 10.79           C
ATOM   3824  CB ASER B  31       4.636   1.385  24.762  0.65 11.07           C
ATOM   3829  OG BSER B  31       5.007   2.545  25.442  0.35 12.74           O
ATOM   3830  OG ASER B  31       5.642   0.393  24.813  0.65 12.96           O
ATOM   3833  C    SER B  31       2.236   1.796  25.263  1.00  8.79           C
ATOM   3834  O    SER B  31       1.624   2.194  24.261  1.00 10.03           O
ATOM   3835  N    CYS B  32       2.006   2.249  26.481  1.00  8.21           N
ATOM   3837  CA   CYS B  32       0.981   3.237  26.755  1.00  8.25           C
ATOM   3839  CB BCYS B  32      -0.398   2.638  26.853  0.35  9.91           C
ATOM   3840  CB ACYS B  32      -0.338   2.497  27.106  0.65  8.79           C
ATOM   3845  SG BCYS B  32      -0.604   1.615  28.261  0.35 14.50           S
ATOM   3846  SG ACYS B  32      -1.274   1.895  25.659  0.65  7.95           S
ATOM   3847  C    CYS B  32       1.399   4.076  27.956  1.00  7.16           C
ATOM   3848  O    CYS B  32       2.526   3.975  28.467  1.00  8.13           O
ATOM   3849  N    THR B  33       0.491   4.947  28.359  1.00  6.54           N
ATOM   3851  CA   THR B  33       0.647   5.783  29.522  1.00  6.41           C
ATOM   3853  CB   THR B  33       0.515   7.251  29.080  1.00  6.34           C
ATOM   3855  OG1  THR B  33       1.515   7.524  28.079  1.00  6.92           O
ATOM   3857  CG2  THR B  33       0.761   8.237  30.220  1.00  6.68           C
ATOM   3861  C    THR B  33      -0.451   5.417  30.520  1.00  6.49           C
ATOM   3862  O    THR B  33      -1.496   4.893  30.137  1.00  6.80           O
ATOM   3863  N    GLY B  34      -0.228   5.715  31.793  1.00  6.76           N
ATOM   3865  CA   GLY B  34      -1.290   5.682  32.779  1.00  6.72           C
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ATOM   3868  C   GLY B  34      -1.039   6.736  33.827  1.00  6.52           C
ATOM   3869  O   GLY B  34      -0.075   7.493  33.760  1.00  6.78           O
ATOM   3870  N   TRP B  35      -1.887   6.753  34.838  1.00  6.86           N
ATOM   3872  CA  TRP B  35      -1.766   7.724  35.904  1.00  7.26           C
ATOM   3874  CB  TRP B  35      -2.492   9.043  35.563  1.00  7.82           C
ATOM   3877  CG  TRP B  35      -3.831   8.901  34.906  1.00  8.11           C
ATOM   3878  CD1 TRP B  35      -4.066   8.555  33.608  1.00  8.12           C
ATOM   3880  NE1 TRP B  35      -5.414   8.580  33.339  1.00  8.93           N
ATOM   3882  CE2 TRP B  35      -6.079   8.965  34.473  1.00  8.81           C
ATOM   3883  CD2 TRP B  35      -5.111   9.181  35.475  1.00  7.96           C
ATOM   3884  CE3 TRP B  35      -5.542   9.590  36.735  1.00  8.75           C
ATOM   3886  CZ3 TRP B  35      -6.887   9.760  36.966  1.00  9.89           C
ATOM   3888  CH2 TRP B  35      -7.814   9.526  35.963  1.00 10.09           C
ATOM   3890  CZ2 TRP B  35      -7.432   9.140  34.705  1.00 10.05           C
ATOM   3892  C   TRP B  35      -2.265   7.119  37.203  1.00  7.17           C
ATOM   3893  O   TRP B  35      -3.305   6.444  37.247  1.00  7.48           O
ATOM   3894  N   MET B  36      -1.514   7.324  38.276  1.00  7.22           N
ATOM   3896  CA  MET B  36      -1.884   6.750  39.562  1.00  7.60           C
ATOM   3898  CB  MET B  36      -0.790   6.983  40.601  1.00  8.12           C
ATOM   3901  CG  MET B  36       0.593   6.429  40.265  1.00  8.68           C
ATOM   3904  SD  MET B  36       0.683   4.684  39.895  1.00  9.14           S
ATOM   3905  CE  MET B  36       0.098   4.015  41.440  1.00  9.93           C
ATOM   3909  C   MET B  36      -3.173   7.378  40.084  1.00  7.70           C
ATOM   3910  O   MET B  36      -3.339   8.603  40.029  1.00  8.47           O
ATOM   3911  N   ILE B  37      -4.055   6.534  40.632  1.00  7.60           N
ATOM   3913  CA  ILE B  37      -5.248   6.992  41.337  1.00  8.62           C
ATOM   3915  CB  ILE B  37      -6.553   6.614  40.591  1.00  8.72           C
ATOM   3917  CG1 ILE B  37      -6.723   5.099  40.438  1.00  9.33           C
ATOM   3920  CD1 ILE B  37      -8.120   4.724  39.928  1.00  9.73           C
ATOM   3924  CG2 ILE B  37      -6.607   7.330  39.261  1.00  9.21           C
ATOM   3928  C   ILE B  37      -5.294   6.519  42.789  1.00  8.85           C
ATOM   3929  O   ILE B  37      -6.214   6.872  43.524  1.00 10.47           O
ATOM   3930  N   GLY B  38      -4.311   5.739  43.210  1.00  9.34           N
ATOM   3932  CA  GLY B  38      -4.205   5.289  44.585  1.00  9.66           C
ATOM   3935  C   GLY B  38      -2.837   4.675  44.794  1.00  9.97           C
ATOM   3936  O   GLY B  38      -1.986   4.723  43.900  1.00 10.35           O
ATOM   3937  N   PRO B  39      -2.597   4.131  45.975  1.00  9.86           N
ATOM   3938  CA  PRO B  39      -1.304   3.498  46.274  1.00 10.14           C
ATOM   3940  CB  PRO B  39      -1.552   2.839  47.634  1.00 10.75           C
ATOM   3943  CG  PRO B  39      -2.545   3.766  48.271  1.00 11.80           C
ATOM   3946  CD  PRO B  39      -3.486   4.139  47.149  1.00 10.25           C
ATOM   3949  C   PRO B  39      -0.830   2.487  45.238  1.00  9.69           C
ATOM   3950  O   PRO B  39       0.366   2.411  44.978  1.00 10.04           O
ATOM   3951  N   LYS B  40      -1.734   1.687  44.702  1.00  9.60           N
ATOM   3953  CA  LYS B  40      -1.328   0.634  43.791  1.00  9.71           C
ATOM   3955  CB  LYS B  40      -1.113  -0.678  44.529  1.00 11.09           C
ATOM   3958  CG  LYS B  40      -2.335  -1.186  45.229  1.00 11.94           C
ATOM   3961  CD  LYS B  40      -2.132  -2.615  45.726  1.00 13.45           C
ATOM   3964  CE  LYS B  40      -0.996  -2.749  46.704  1.00 14.20           C
ATOM   3967  NZ  LYS B  40      -0.976  -4.121  47.344  1.00 15.10           N
ATOM   3971  C   LYS B  40      -2.284   0.467  42.617  1.00  8.70           C
ATOM   3972  O   LYS B  40      -2.366  -0.617  42.060  1.00  9.87           O
ATOM   3973  N   THR B  41      -2.985   1.532  42.227  1.00  8.11           N
ATOM   3975  CA  THR B  41      -3.939   1.455  41.125  1.00  8.14           C
ATOM   3977  CB  THR B  41      -5.375   1.586  41.663  1.00  8.25           C
ATOM   3979  OG1 THR B  41      -5.572   0.652  42.741  1.00  9.37           O
ATOM   3981  CG2 THR B  41      -6.399   1.262  40.576  1.00  9.16           C
ATOM   3985  C   THR B  41      -3.641   2.556  40.130  1.00  7.63           C
ATOM   3986  O   THR B  41      -3.476   3.711  40.515  1.00  8.27           O
ATOM   3987  N   VAL B  42      -3.590   2.160  38.861  1.00  7.48           N
ATOM   3989  CA  VAL B  42      -3.271   3.007  37.732  1.00  7.56           C
ATOM   3991  CB  VAL B  42      -2.122   2.378  36.911  1.00  7.80           C
ATOM   3993  CG1 VAL B  42      -1.745   3.260  35.729  1.00  8.94           C
ATOM   3997  CG2 VAL B  42      -0.914   2.085  37.763  1.00  9.62           C
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ATOM   4001  C   VAL B  42      -4.491   3.072  36.818  1.00  7.34           C
ATOM   4002  O   VAL B  42      -5.024   2.044  36.433  1.00  9.14           O
ATOM   4003  N   ALA B  43      -4.918   4.274  36.432  1.00  7.37           N
ATOM   4005  CA  ALA B  43      -5.911   4.442  35.377  1.00  7.20           C
ATOM   4007  CB  ALA B  43      -6.711   5.713  35.603  1.00  7.51           C
ATOM   4011  C   ALA B  43      -5.214   4.503  34.017  1.00  7.00           C
ATOM   4012  O   ALA B  43      -4.129   5.081  33.886  1.00  7.26           O
ATOM   4013  N   THR B  44      -5.836   3.904  33.019  1.00  6.97           N
ATOM   4015  CA  THR B  44      -5.286   3.897  31.670  1.00  7.04           C
ATOM   4017  CB  THR B  44      -4.160   2.834  31.570  1.00  7.41           C
ATOM   4019  OG1 THR B  44      -3.485   2.938  30.303  1.00  7.54           O
ATOM   4021  CG2 THR B  44      -4.692   1.413  31.698  1.00  7.72           C
ATOM   4025  C   THR B  44      -6.413   3.683  30.656  1.00  6.99           C
ATOM   4026  O   THR B  44      -7.596   3.731  30.998  1.00  7.52           O
ATOM   4027  N   ALA B  45      -6.048   3.485  29.395  1.00  7.00           N
ATOM   4029  CA  ALA B  45      -7.003   3.149  28.349  1.00  7.12           C
ATOM   4031  CB  ALA B  45      -6.479   3.579  26.979  1.00  7.53           C
ATOM   4035  C   ALA B  45      -7.281   1.644  28.351  1.00  7.28           C
ATOM   4036  O   ALA B  45      -6.370   0.833  28.543  1.00  8.36           O
ATOM   4037  N   GLY B  46      -8.529   1.256  28.120  1.00  7.41           N
ATOM   4039  CA  GLY B  46      -8.874  -0.156  28.014  1.00  7.78           C
ATOM   4042  C   GLY B  46      -8.106  -0.884  26.933  1.00  7.87           C
ATOM   4043  O   GLY B  46      -7.669  -2.017  27.135  1.00  8.48           O
ATOM   4044  N   HIS B  47      -7.940  -0.234  25.783  1.00  7.88           N
ATOM   4046  CA  HIS B  47      -7.288  -0.893  24.672  1.00  8.40           C
ATOM   4048  CB  HIS B  47      -7.524  -0.133  23.362  1.00  8.56           C
ATOM   4051  CG  HIS B  47      -6.718   1.122  23.182  1.00  7.89           C
ATOM   4052  ND1 HIS B  47      -7.280   2.381  23.233  1.00  8.37           N
ATOM   4054  CE1 HIS B  47      -6.356   3.284  22.954  1.00  8.17           C
ATOM   4056  NE2 HIS B  47      -5.209   2.668  22.753  1.00  8.05           N
ATOM   4058  CD2 HIS B  47      -5.409   1.313  22.884  1.00  7.79           C
ATOM   4060  C   HIS B  47      -5.808  -1.162  24.909  1.00  8.34           C
ATOM   4061  O   HIS B  47      -5.198  -1.909  24.160  1.00  9.86           O
ATOM   4062  N   CYS B  48      -5.235  -0.537  25.933  1.00  7.91           N
ATOM   4064  CA  CYS B  48      -3.850  -0.803  26.311  1.00  8.43           C
ATOM   4066  CB  CYS B  48      -3.317   0.340  27.164  1.00  9.43           C
ATOM   4069  SG  CYS B  48      -3.197   1.908  26.286  1.00 11.14           S
ATOM   4070  C   CYS B  48      -3.671  -2.102  27.099  1.00  8.41           C
ATOM   4071  O   CYS B  48      -2.553  -2.599  27.197  1.00  9.30           O
ATOM   4072  N   ILE B  49      -4.758  -2.622  27.679  1.00  8.25           N
ATOM   4074  CA  ILE B  49      -4.680  -3.771  28.589  1.00  8.11           C
ATOM   4076  CB  ILE B  49      -4.931  -3.327  30.049  1.00  8.38           C
ATOM   4078  CG1 ILE B  49      -6.349  -2.791  30.254  1.00  8.89           C
ATOM   4081  CD1 ILE B  49      -6.631  -2.365  31.696  1.00  9.33           C
ATOM   4085  CG2 ILE B  49      -3.871  -2.314  30.454  1.00  9.04           C
ATOM   4089  C   ILE B  49      -5.574  -4.945  28.224  1.00  8.36           C
ATOM   4090  O   ILE B  49      -5.385  -6.015  28.774  1.00  8.42           O
ATOM   4091  N   TYR B  50      -6.527  -4.765  27.313  1.00  8.78           N
ATOM   4093  CA  TYR B  50      -7.397  -5.847  26.876  1.00  9.04           C
ATOM   4095  CB  TYR B  50      -8.752  -5.812  27.602  1.00  9.41           C
ATOM   4098  CG  TYR B  50      -9.689  -6.905  27.142  1.00 10.04           C
ATOM   4099  CD1 TYR B  50     -10.686  -6.650  26.211  1.00 10.86           C
ATOM   4101  CE1 TYR B  50     -11.534  -7.668  25.770  1.00 11.77           C
ATOM   4103  CZ  TYR B  50     -11.372  -8.951  26.279  1.00 11.98           C
ATOM   4104  OH  TYR B  50     -12.188  -9.993  25.878  1.00 14.06           O
ATOM   4106  CE2 TYR B  50     -10.394  -9.208  27.210  1.00 11.89           C
ATOM   4108  CD2 TYR B  50      -9.549  -8.200  27.615  1.00 10.91           C
ATOM   4110  C   TYR B  50      -7.585  -5.731  25.363  1.00  9.64           C
ATOM   4111  O   TYR B  50      -8.007  -4.678  24.858  1.00 10.03           O
ATOM   4112  N   ASP B  51      -7.221  -6.802  24.663  1.00 10.47           N
ATOM   4114  CA  ASP B  51      -7.291  -6.906  23.220  1.00 12.23           C
ATOM   4116  CB BASP B  51      -6.107  -7.742  22.729  0.35 12.66           C
ATOM   4117  CB AASP B  51      -6.122  -7.695  22.640  0.65 13.16           C
ATOM   4122  CG BASP B  51      -6.080  -7.888  21.234  0.35 13.82           C
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ATOM   4123  CG AASP B  51      -6.149  -7.713  21.131  0.65 15.14           C
ATOM   4124  OD1BASP B  51      -6.122  -9.033  20.747  0.35 14.80           O
ATOM   4125  OD1AASP B  51      -5.098  -7.505  20.497  0.65 16.90           O
ATOM   4126  OD2BASP B  51      -6.018  -6.909  20.468  0.35 15.44           O
ATOM   4127  OD2AASP B  51      -7.200  -7.900  20.492  0.65 16.43           O
ATOM   4128  C    ASP B  51      -8.601  -7.577  22.843  1.00 11.68           C
ATOM   4129  O    ASP B  51      -8.809  -8.770  23.089  1.00 12.14           O
ATOM   4130  N    THR B  52      -9.484  -6.811  22.224  1.00 12.82           N
ATOM   4132  CA   THR B  52     -10.821  -7.311  21.944  1.00 14.29           C
ATOM   4134  CB   THR B  52     -11.794  -6.158  21.621  1.00 15.31           C
ATOM   4136  OG1  THR B  52     -11.342  -5.436  20.473  1.00 17.85           O
ATOM   4138  CG2  THR B  52     -11.813  -5.133  22.748  1.00 15.84           C
ATOM   4142  C    THR B  52     -10.849  -8.374  20.842  1.00 15.07           C
ATOM   4143  O    THR B  52     -11.736  -9.221  20.836  1.00 16.91           O
ATOM   4144  N    SER B  53      -9.900  -8.338  19.911  1.00 15.21           N
ATOM   4146  CA   SER B  53      -9.869  -9.326  18.824  1.00 15.87           C
ATOM   4148  CB BSER B  53      -8.908  -8.886  17.708  0.35 16.21           C
ATOM   4149  CB ASER B  53      -8.859  -8.903  17.756  0.65 16.72           C
ATOM   4154  OG BSER B  53      -7.569  -8.772  18.157  0.35 17.00           O
ATOM   4155  OG ASER B  53      -8.752  -9.892  16.748  0.65 18.99           O
ATOM   4158  C    SER B  53      -9.530 -10.736  19.309  1.00 15.03           C
ATOM   4159  O    SER B  53     -10.178 -11.722  18.919  1.00 14.93           O
ATOM   4160  N    SER B  54      -8.511 -10.836  20.153  1.00 14.11           N
ATOM   4162  CA   SER B  54      -8.082 -12.117  20.691  1.00 13.76           C
ATOM   4164  CB   SER B  54      -6.585 -12.082  20.984  1.00 14.63           C
ATOM   4167  OG   SER B  54      -6.302 -11.212  22.069  1.00 15.48           O
ATOM   4169  C    SER B  54      -8.830 -12.497  21.955  1.00 12.67           C
ATOM   4170  O    SER B  54      -8.716 -13.624  22.416  1.00 13.34           O
ATOM   4171  N    GLY B  55      -9.564 -11.539  22.518  1.00 12.60           N
ATOM   4173  CA   GLY B  55     -10.337 -11.766  23.724  1.00 12.43           C
ATOM   4176  C    GLY B  55      -9.474 -11.987  24.936  1.00 11.80           C
ATOM   4177  O    GLY B  55      -9.834 -12.737  25.833  1.00 12.09           O
ATOM   4178  N    SER B  56      -8.333 -11.313  24.993  1.00 12.30           N
ATOM   4180  CA   SER B  56      -7.404 -11.563  26.071  1.00 12.22           C
ATOM   4182  CB   SER B  56      -6.277 -12.470  25.600  1.00 13.33           C
ATOM   4185  OG   SER B  56      -5.511 -11.840  24.607  1.00 17.47           O
ATOM   4187  C    SER B  56      -6.813 -10.288  26.619  1.00 10.81           C
ATOM   4188  O    SER B  56      -6.567  -9.310  25.907  1.00 10.50           O
ATOM   4189  N    PHE B  57      -6.573 -10.325  27.916  1.00  9.99           N
ATOM   4191  CA   PHE B  57      -5.790  -9.301  28.562  1.00  9.43           C
ATOM   4193  CB   PHE B  57      -5.887  -9.455  30.080  1.00 10.07           C
ATOM   4196  CG   PHE B  57      -7.232  -9.069  30.620  1.00 10.41           C
ATOM   4197  CD1  PHE B  57      -7.527  -7.744  30.869  1.00 10.08           C
ATOM   4199  CE1  PHE B  57      -8.774  -7.363  31.333  1.00 11.19           C
ATOM   4201  CZ   PHE B  57      -9.751  -8.313  31.532  1.00 12.88           C
ATOM   4203  CE2  PHE B  57      -9.476  -9.645  31.264  1.00 13.00           C
ATOM   4205  CD2  PHE B  57      -8.230 -10.020  30.810  1.00 12.20           C
ATOM   4207  C    PHE B  57      -4.347  -9.410  28.102  1.00  9.19           C
ATOM   4208  O    PHE B  57      -3.877 -10.475  27.678  1.00 10.24           O
ATOM   4209  N    ALA B  58      -3.643  -8.288  28.189  1.00  9.20           N
ATOM   4211  CA   ALA B  58      -2.202  -8.292  28.075  1.00  9.09           C
ATOM   4213  CB   ALA B  58      -1.664  -6.887  28.322  1.00  9.63           C
ATOM   4217  C    ALA B  58      -1.601  -9.247  29.090  1.00  9.25           C
ATOM   4218  O    ALA B  58      -2.213  -9.573  30.105  1.00  9.38           O
ATOM   4219  N    GLY B  59      -0.371  -9.666  28.838  1.00  9.59           N
ATOM   4221  CA   GLY B  59       0.444 -10.276  29.857  1.00  9.95           C
ATOM   4224  C    GLY B  59       0.793  -9.242  30.908  1.00  9.76           C
ATOM   4225  O    GLY B  59       0.308  -8.099  30.891  1.00 10.29           O
ATOM   4226  N    THR B  60       1.637  -9.646  31.834  1.00 10.02           N
ATOM   4228  CA   THR B  60       2.060  -8.759  32.898  1.00 10.25           C
ATOM   4230  CB   THR B  60       3.107  -9.463  33.740  1.00 11.48           C
ATOM   4232  OG1  THR B  60       2.519 -10.662  34.262  1.00 13.35           O
ATOM   4234  CG2  THR B  60       3.526  -8.622  34.941  1.00 12.09           C
ATOM   4238  C    THR B  60       2.629  -7.471  32.338  1.00  9.81           C
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ATOM   4239  O   THR B  60       3.465  -7.498  31.441  1.00 10.64           O
ATOM   4240  N   ALA B  61       2.176  -6.351  32.884  1.00  9.32           N
ATOM   4242  CA  ALA B  61       2.677  -5.044  32.503  1.00  9.32           C
ATOM   4244  CB  ALA B  61       1.568  -3.981  32.587  1.00  9.62           C
ATOM   4248  C   ALA B  61       3.837  -4.632  33.385  1.00  8.92           C
ATOM   4249  O   ALA B  61       3.876  -4.954  34.567  1.00 10.09           O
ATOM   4250  N   THR B  62       4.756  -3.882  32.793  1.00  9.06           N
ATOM   4252  CA  THR B  62       5.844  -3.224  33.497  1.00  9.56           C
ATOM   4254  CB  THR B  62       7.159  -3.456  32.762  1.00 10.57           C
ATOM   4256  OG1 THR B  62       7.423  -4.870  32.721  1.00 11.83           O
ATOM   4258  CG2 THR B  62       8.326  -2.808  33.497  1.00 12.14           C
ATOM   4262  C   THR B  62       5.495  -1.745  33.556  1.00  8.59           C
ATOM   4263  O   THR B  62       5.334  -1.089  32.521  1.00  9.17           O
ATOM   4264  N   VAL B  63       5.359  -1.225  34.771  1.00  8.26           N
ATOM   4266  CA  VAL B  63       4.826   0.118  35.013  1.00  8.04           C
ATOM   4268  CB  VAL B  63       3.546   0.039  35.861  1.00  8.65           C
ATOM   4270  CG1 VAL B  63       3.023   1.431  36.176  1.00  9.71           C
ATOM   4274  CG2 VAL B  63       2.478  -0.794  35.150  1.00  9.51           C
ATOM   4278  C   VAL B  63       5.891   0.959  35.693  1.00  7.95           C
ATOM   4279  O   VAL B  63       6.369   0.597  36.771  1.00  8.82           O
ATOM   4280  N   SER B  64       6.254   2.083  35.085  1.00  7.68           N
ATOM   4282  CA  SER B  64       7.393   2.863  35.515  1.00  8.03           C
ATOM   4284  CB  SER B  64       8.499   2.805  34.462  1.00  8.70           C
ATOM   4287  OG  SER B  64       8.898   1.469  34.228  1.00  9.66           O
ATOM   4289  C   SER B  64       6.965   4.306  35.757  1.00  7.95           C
ATOM   4290  O   SER B  64       6.893   5.116  34.823  1.00  7.83           O
ATOM   4291  N   PRO B  65       6.648   4.658  37.004  1.00  8.11           N
ATOM   4292  CA  PRO B  65       6.226   6.028  37.301  1.00  8.10           C
ATOM   4294  CB  PRO B  65       5.859   5.970  38.795  1.00  8.49           C
ATOM   4297  CG  PRO B  65       5.584   4.520  39.054  1.00  8.49           C
ATOM   4300  CD  PRO B  65       6.600   3.807  38.204  1.00  8.68           C
ATOM   4303  C   PRO B  65       7.344   7.027  37.057  1.00  8.00           C
ATOM   4304  O   PRO B  65       8.483   6.807  37.481  1.00  8.46           O
ATOM   4305  N   GLY B  66       7.038   8.127  36.383  1.00  7.75           N
ATOM   4307  CA  GLY B  66       8.034   9.166  36.186  1.00  8.40           C
ATOM   4310  C   GLY B  66       9.266   8.699  35.428  1.00  8.24           C
ATOM   4311  O   GLY B  66      10.346   9.265  35.586  1.00  8.86           O
ATOM   4312  N   ARG B  67       9.123   7.685  34.585  1.00  8.08           N
ATOM   4314  CA  ARG B  67      10.223   7.252  33.745  1.00  8.11           C
ATOM   4316  CB  ARG B  67       9.753   6.160  32.802  1.00  8.27           C
ATOM   4319  CG  ARG B  67      10.864   5.568  31.971  1.00  8.88           C
ATOM   4322  CD  ARG B  67      10.435   4.444  31.086  1.00  8.89           C
ATOM   4325  NE  ARG B  67      11.498   4.135  30.142  1.00  9.16           N
ATOM   4327  CZ  ARG B  67      11.404   3.282  29.149  1.00 10.30           C
ATOM   4328  NH1 ARG B  67      12.410   3.169  28.296  1.00 11.25           N
ATOM   4331  NH2 ARG B  67      10.320   2.541  29.004  1.00 12.36           N
ATOM   4334  C   ARG B  67      10.750   8.429  32.946  1.00  8.11           C
ATOM   4335  O   ARG B  67       9.983   9.254  32.462  1.00  8.22           O
ATOM   4336  N   ASN B  68      12.070   8.472  32.783  1.00  8.17           N
ATOM   4338  CA  ASN B  68      12.720   9.484  31.970  1.00  8.77           C
ATOM   4340  CB  ASN B  68      13.312  10.573  32.848  1.00  9.40           C
ATOM   4343  CG  ASN B  68      13.931  11.660  32.023  1.00 10.39           C
ATOM   4344  OD1 ASN B  68      13.349  12.050  31.010  1.00 11.79           O
ATOM   4345  ND2 ASN B  68      15.136  12.110  32.385  1.00 12.51           N
ATOM   4348  C   ASN B  68      13.812   8.863  31.104  1.00  9.03           C
ATOM   4349  O   ASN B  68      14.994   8.879  31.455  1.00  9.74           O
ATOM   4350  N   GLY B  69      13.405   8.293  29.977  1.00  9.60           N
ATOM   4352  CA  GLY B  69      14.329   7.682  29.037  1.00  9.82           C
ATOM   4355  C   GLY B  69      14.763   6.335  29.549  1.00  9.55           C
ATOM   4356  O   GLY B  69      13.946   5.419  29.628  1.00 10.48           O
ATOM   4357  N   THR B  70      16.040   6.194  29.885  1.00  9.50           N
ATOM   4359  CA  THR B  70      16.516   4.977  30.529  1.00 10.01           C
ATOM   4361  CB  THR B  70      17.775   4.427  29.839  1.00 10.64           C
ATOM   4363  OG1 THR B  70      18.745   5.471  29.679  1.00 11.68           O
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ATOM   4365  CG2 THR B  70      17.437   3.934  28.436  1.00 11.69           C
ATOM   4369  C   THR B  70      16.747   5.185  32.024  1.00 10.48           C
ATOM   4370  O   THR B  70      17.362   4.357  32.689  1.00 11.63           O
ATOM   4371  N   SER B  71      16.214   6.274  32.558  1.00 10.58           N
ATOM   4373  CA  SER B  71      16.175   6.510  33.992  1.00 10.32           C
ATOM   4375  CB  SER B  71      16.437   7.969  34.309  1.00 10.45           C
ATOM   4378  OG  SER B  71      17.669   8.393  33.780  1.00 11.02           O
ATOM   4380  C   SER B  71      14.821   6.139  34.562  1.00  9.95           C
ATOM   4381  O   SER B  71      13.775   6.496  34.006  1.00 10.07           O
ATOM   4382  N   TYR B  72      14.853   5.470  35.710  1.00  9.77           N
ATOM   4384  CA  TYR B  72      13.665   4.953  36.370  1.00  9.83           C
ATOM   4386  CB  TYR B  72      13.637   3.420  36.298  1.00 10.11           C
ATOM   4389  CG  TYR B  72      13.491   2.884  34.890  1.00 10.38           C
ATOM   4390  CD1 TYR B  72      12.261   2.467  34.422  1.00 10.73           C
ATOM   4392  CE1 TYR B  72      12.112   1.963  33.142  1.00 11.24           C
ATOM   4394  CZ  TYR B  72      13.200   1.895  32.301  1.00 11.23           C
ATOM   4395  OH  TYR B  72      13.014   1.381  31.041  1.00 12.73           O
ATOM   4397  CE2 TYR B  72      14.442   2.316  32.741  1.00 11.74           C
ATOM   4399  CD2 TYR B  72      14.581   2.804  34.018  1.00 11.15           C
ATOM   4401  C   TYR B  72      13.739   5.443  37.815  1.00 10.00           C
ATOM   4402  O   TYR B  72      14.125   4.683  38.712  1.00 10.50           O
ATOM   4403  N   PRO B  73      13.426   6.715  38.070  1.00 10.19           N
ATOM   4404  CA  PRO B  73      13.605   7.254  39.425  1.00 10.57           C
ATOM   4406  CB  PRO B  73      13.195   8.719  39.285  1.00 10.64           C
ATOM   4409  CG  PRO B  73      12.351   8.766  38.059  1.00 10.69           C
ATOM   4412  CD  PRO B  73      12.927   7.742  37.134  1.00 10.07           C
ATOM   4415  C   PRO B  73      12.778   6.561  40.497  1.00 10.59           C
ATOM   4416  O   PRO B  73      13.139   6.627  41.664  1.00 12.17           O
ATOM   4417  N   TYR B  74      11.692   5.916  40.097  1.00 10.13           N
ATOM   4419  CA  TYR B  74      10.834   5.165  41.004  1.00 10.86           C
ATOM   4421  CB  TYR B  74       9.425   5.767  41.038  1.00 10.82           C
ATOM   4424  CG  TYR B  74       9.500   7.222  41.399  1.00 10.36           C
ATOM   4425  CD1 TYR B  74       9.391   8.194  40.416  1.00 10.82           C
ATOM   4427  CE1 TYR B  74       9.519   9.518  40.701  1.00 11.59           C
ATOM   4429  CZ  TYR B  74       9.748   9.915  41.996  1.00 11.91           C
ATOM   4430  OH  TYR B  74       9.863  11.261  42.253  1.00 14.01           O
ATOM   4432  CE2 TYR B  74       9.864   8.972  43.005  1.00 12.35           C
ATOM   4434  CD2 TYR B  74       9.752   7.632  42.700  1.00 11.52           C
ATOM   4436  C   TYR B  74      10.788   3.696  40.635  1.00 11.39           C
ATOM   4437  O   TYR B  74       9.849   2.993  41.013  1.00 12.84           O
ATOM   4438  N   GLY B  75      11.820   3.222  39.939  1.00 10.85           N
ATOM   4440  CA  GLY B  75      11.872   1.851  39.479  1.00 10.79           C
ATOM   4443  C   GLY B  75      10.764   1.505  38.505  1.00 10.56           C
ATOM   4444  O   GLY B  75      10.129   2.370  37.891  1.00 10.85           O
ATOM   4445  N   SER B  76      10.563   0.202  38.377  1.00 10.90           N
ATOM   4447  CA  SER B  76       9.489  -0.367  37.607  1.00 11.52           C
ATOM   4449  CB BSER B  76      10.053  -1.085  36.386  0.35 11.19           C
ATOM   4450  CB ASER B  76       9.998  -0.975  36.309  0.65 13.32           C
ATOM   4455  OG BSER B  76      10.704  -0.188  35.508  0.35  7.99           O
ATOM   4456  OG ASER B  76      10.880  -2.042  36.529  0.65 17.36           O
ATOM   4459  C   SER B  76       8.802  -1.393  38.474  1.00 11.22           C
ATOM   4460  O   SER B  76       9.444  -2.102  39.264  1.00 12.58           O
ATOM   4461  N   VAL B  77       7.489  -1.472  38.325  1.00 10.56           N
ATOM   4463  CA  VAL B  77       6.668  -2.352  39.116  1.00 10.65           C
ATOM   4465  CB BVAL B  77       5.793  -1.531  40.080  0.35 10.56           C
ATOM   4466  CB AVAL B  77       5.843  -1.555  40.151  0.65 11.99           C
ATOM   4469  CG1BVAL B  77       4.837  -2.397  40.810  0.35  8.39           C
ATOM   4470  CG1AVAL B  77       6.704  -0.441  40.775  0.65 12.64           C
ATOM   4477  CG2BVAL B  77       6.661  -0.843  41.119  0.35 11.33           C
ATOM   4478  CG2AVAL B  77       4.627  -0.943  39.578  0.65 12.49           C
ATOM   4485  C   VAL B  77       5.801  -3.183  38.174  1.00  9.91           C
ATOM   4486  O   VAL B  77       5.303  -2.699  37.163  1.00 12.13           O
ATOM   4487  N   LYS B  78       5.596  -4.440  38.500  1.00 11.93           N
ATOM   4489  CA  LYS B  78       4.790  -5.315  37.664  1.00 12.70           C
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ATOM   4491  CB  LYS B  78       5.236  -6.767  37.809  1.00 13.36           C
ATOM   4494  CG  LYS B  78       6.666  -7.034  37.399  1.00 15.87           C
ATOM   4497  CD  LYS B  78       6.906  -6.736  35.938  1.00 17.52           C
ATOM   4500  CE  LYS B  78       8.294  -7.176  35.540  1.00 19.94           C
ATOM   4503  NZ  LYS B  78       8.671  -6.739  34.188  1.00 22.53           N
ATOM   4507  C   LYS B  78       3.338  -5.215  38.065  1.00 12.78           C
ATOM   4508  O   LYS B  78       3.035  -5.045  39.243  1.00 14.43           O
ATOM   4509  N   SER B  79       2.436  -5.360  37.098  1.00 12.03           N
ATOM   4511  CA  SER B  79       1.017  -5.509  37.378  1.00 11.74           C
ATOM   4513  CB  SER B  79       0.196  -5.437  36.090  1.00 11.51           C
ATOM   4516  OG  SER B  79       0.508  -6.477  35.178  1.00 10.77           O
ATOM   4518  C   SER B  79       0.722  -6.833  38.044  1.00 11.16           C
ATOM   4519  O   SER B  79       1.441  -7.826  37.856  1.00 12.07           O
ATOM   4520  N   THR B  80      -0.360  -6.849  38.804  1.00 10.75           N
ATOM   4522  CA  THR B  80      -0.923  -8.093  39.298  1.00 11.21           C
ATOM   4524  CB  THR B  80      -1.164  -8.015  40.807  1.00 11.49           C
ATOM   4526  OG1 THR B  80      -1.989  -6.887  41.124  1.00 12.77           O
ATOM   4528  CG2 THR B  80       0.154  -7.823  41.547  1.00 11.88           C
ATOM   4532  C   THR B  80      -2.196  -8.485  38.578  1.00 11.33           C
ATOM   4533  O   THR B  80      -2.490  -9.682  38.478  1.00 12.80           O
ATOM   4534  N   ARG B  81      -2.959  -7.489  38.114  1.00 11.02           N
ATOM   4536  CA  ARG B  81      -4.210  -7.752  37.427  1.00 10.96           C
ATOM   4538  CB  ARG B  81      -5.240  -8.338  38.374  1.00 11.73           C
ATOM   4541  CG  ARG B  81      -5.626  -7.375  39.459  1.00 11.26           C
ATOM   4544  CD  ARG B  81      -6.558  -7.993  40.419  1.00 13.29           C
ATOM   4547  NE  ARG B  81      -6.874  -7.102  41.525  1.00 14.74           N
ATOM   4549  CZ  ARG B  81      -7.891  -7.291  42.357  1.00 13.37           C
ATOM   4550  NH1 ARG B  81      -8.139  -6.424  43.336  1.00 11.04           N
ATOM   4553  NH2 ARG B  81      -8.704  -8.320  42.185  1.00 16.83           N
ATOM   4556  C   ARG B  81      -4.748  -6.458  36.824  1.00  9.86           C
ATOM   4557  O   ARG B  81      -4.234  -5.348  37.074  1.00 10.32           O
ATOM   4558  N   TYR B  82      -5.781  -6.619  36.013  1.00  9.05           N
ATOM   4560  CA  TYR B  82      -6.392  -5.564  35.243  1.00  8.45           C
ATOM   4562  CB  TYR B  82      -6.236  -5.882  33.761  1.00  8.46           C
ATOM   4565  CG  TYR B  82      -4.815  -5.913  33.273  1.00  8.62           C
ATOM   4566  CD1 TYR B  82      -4.012  -4.791  33.367  1.00  9.06           C
ATOM   4568  CE1 TYR B  82      -2.711  -4.804  32.888  1.00  9.18           C
ATOM   4570  CZ  TYR B  82      -2.202  -5.950  32.310  1.00  8.79           C
ATOM   4571  OH  TYR B  82      -0.907  -5.894  31.850  1.00  9.78           O
ATOM   4573  CE2 TYR B  82      -2.990  -7.081  32.209  1.00  9.01           C
ATOM   4575  CD2 TYR B  82      -4.284  -7.053  32.688  1.00  9.11           C
ATOM   4577  C   TYR B  82      -7.886  -5.476  35.560  1.00  8.75           C
ATOM   4578  O   TYR B  82      -8.513  -6.470  35.949  1.00  9.58           O
ATOM   4579  N   PHE B  83      -8.447  -4.290  35.362  1.00  8.61           N
ATOM   4581  CA  PHE B  83      -9.874  -4.032  35.444  1.00  8.66           C
ATOM   4583  CB  PHE B  83     -10.228  -3.092  36.585  1.00  9.00           C
ATOM   4586  CG  PHE B  83      -9.748  -3.516  37.936  1.00  9.24           C
ATOM   4587  CD1 PHE B  83      -8.475  -3.177  38.366  1.00 10.26           C
ATOM   4589  CE1 PHE B  83      -8.059  -3.502  39.639  1.00 11.44           C
ATOM   4591  CZ  PHE B  83      -8.911  -4.173  40.501  1.00 12.62           C
ATOM   4593  CE2 PHE B  83     -10.177  -4.495  40.104  1.00 12.07           C
ATOM   4595  CD2 PHE B  83     -10.604  -4.166  38.823  1.00 10.68           C
ATOM   4597  C   PHE B  83     -10.298  -3.339  34.160  1.00  8.76           C
ATOM   4598  O   PHE B  83      -9.630  -2.409  33.699  1.00  8.65           O
ATOM   4599  N   ILE B  84     -11.421  -3.768  33.598  1.00  8.84           N
ATOM   4601  CA  ILE B  84     -12.048  -3.068  32.478  1.00  9.12           C
ATOM   4603  CB  ILE B  84     -11.734  -3.740  31.118  1.00  9.53           C
ATOM   4605  CG1 ILE B  84     -12.103  -5.225  31.124  1.00 10.28           C
ATOM   4608  CD1 ILE B  84     -11.973  -5.909  29.791  1.00 12.10           C
ATOM   4612  CG2 ILE B  84     -10.281  -3.522  30.746  1.00 10.12           C
ATOM   4616  C   ILE B  84     -13.552  -3.018  32.691  1.00  8.87           C
ATOM   4617  O   ILE B  84     -14.134  -3.904  33.327  1.00  9.55           O
ATOM   4618  N   PRO B  85     -14.198  -2.004  32.131  1.00  9.08           N
ATOM   4619  CA  PRO B  85     -15.660  -1.982  32.154  1.00  9.52           C
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ATOM   4621  CB  PRO B  85     -15.984  -0.561  31.686  1.00 10.01           C
ATOM   4624  CG  PRO B  85     -14.849  -0.235  30.745  1.00  9.80           C
ATOM   4627  CD  PRO B  85     -13.642  -0.866  31.371  1.00  9.00           C
ATOM   4630  C   PRO B  85     -16.212  -3.010  31.176  1.00 10.10           C
ATOM   4631  O   PRO B  85     -15.561  -3.364  30.210  1.00 10.32           O
ATOM   4632  N   SER B  86     -17.437  -3.459  31.407  1.00 11.25           N
ATOM   4634  CA  SER B  86     -18.073  -4.415  30.502  1.00 12.53           C
ATOM   4636  CB BSER B  86     -19.506  -4.715  30.963  0.35 13.18           C
ATOM   4637  CB ASER B  86     -19.476  -4.789  30.986  0.65 13.91           C
ATOM   4642  OG BSER B  86     -19.544  -5.098  32.327  0.35 14.94           O
ATOM   4643  OG ASER B  86     -20.279  -3.644  31.135  0.65 17.06           O
ATOM   4646  C   SER B  86     -18.116  -3.886  29.071  1.00 12.33           C
ATOM   4647  O   SER B  86     -17.957  -4.654  28.127  1.00 13.62           O
ATOM   4648  N   GLY B  87     -18.305  -2.578  28.911  1.00 12.00           N
ATOM   4650  CA  GLY B  87     -18.365  -1.984  27.589  1.00 12.48           C
ATOM   4653  C   GLY B  87     -17.076  -2.129  26.808  1.00 12.31           C
ATOM   4654  O   GLY B  87     -17.114  -2.175  25.583  1.00 14.16           O
ATOM   4655  N   TRP B  88     -15.931  -2.192  27.495  1.00 11.78           N
ATOM   4657  CA  TRP B  88     -14.658  -2.408  26.804  1.00 11.93           C
ATOM   4659  CB  TRP B  88     -13.432  -1.778  27.501  1.00 11.63           C
ATOM   4662  CG  TRP B  88     -12.253  -1.984  26.598  1.00 10.61           C
ATOM   4663  CD1 TRP B  88     -11.202  -2.843  26.769  1.00 10.04           C
ATOM   4665  NE1 TRP B  88     -10.404  -2.843  25.652  1.00 10.00           N
ATOM   4667  CE2 TRP B  88     -10.932  -1.976  24.733  1.00  9.81           C
ATOM   4668  CD2 TRP B  88     -12.106  -1.434  25.292  1.00 10.13           C
ATOM   4669  CE3 TRP B  88     -12.838  -0.519  24.539  1.00 11.31           C
ATOM   4671  CZ3 TRP B  88     -12.403  -0.194  23.276  1.00 12.74           C
ATOM   4673  CH2 TRP B  88     -11.247  -0.752  22.749  1.00 12.39           C
ATOM   4675  CZ2 TRP B  88     -10.504  -1.659  23.451  1.00 11.10           C
ATOM   4677  C   TRP B  88     -14.384  -3.874  26.554  1.00 13.20           C
ATOM   4678  O   TRP B  88     -13.795  -4.228  25.544  1.00 14.61           O
ATOM   4679  N   ARG B  89     -14.818  -4.742  27.456  1.00 14.97           N
ATOM   4681  CA  ARG B  89     -14.786  -6.157  27.135  1.00 17.45           C
ATOM   4683  CB  ARG B  89     -15.489  -6.978  28.216  1.00 18.59           C
ATOM   4686  CG  ARG B  89     -14.972  -8.407  28.352  1.00 20.17           C
ATOM   4689  CD  ARG B  89     -15.609  -9.163  29.496  1.00 22.60           C
ATOM   4692  NE  ARG B  89     -15.033  -8.796  30.790  1.00 24.39           N
ATOM   4694  CZ  ARG B  89     -13.948  -9.349  31.330  1.00 25.64           C
ATOM   4695  NH1 ARG B  89     -13.279 -10.314  30.701  1.00 26.15           N
ATOM   4698  NH2 ARG B  89     -13.524  -8.931  32.516  1.00 26.48           N
ATOM   4701  C   ARG B  89     -15.423  -6.339  25.731  1.00 19.17           C
ATOM   4702  O   ARG B  89     -15.043  -7.254  24.999  1.00 20.41           O
ATOM   4703  N   SER B  90     -16.345  -5.436  25.357  1.00 20.72           N
ATOM   4705  CA  SER B  90     -16.995  -5.405  24.034  1.00 21.67           C
ATOM   4707  CB  SER B  90     -18.412  -4.837  24.189  1.00 22.17           C
ATOM   4710  OG  SER B  90     -19.158  -5.584  25.125  1.00 23.91           O
ATOM   4712  C   SER B  90     -16.267  -4.630  22.917  1.00 21.74           C
ATOM   4713  O   SER B  90     -16.614  -4.789  21.746  1.00 23.22           O
ATOM   4714  N   GLY B  91     -15.307  -3.771  23.253  1.00 21.01           N
ATOM   4716  CA  GLY B  91     -14.547  -3.027  22.258  1.00 20.45           C
ATOM   4719  C   GLY B  91     -15.224  -1.724  21.881  1.00 20.20           C
ATOM   4720  O   GLY B  91     -14.868  -1.062  20.893  1.00 20.84           O
ATOM   4721  N   ASN B  92     -16.222  -1.355  22.669  1.00 19.28           N
ATOM   4723  CA  ASN B  92     -16.957  -0.132  22.417  1.00 18.38           C
ATOM   4725  CB  ASN B  92     -18.294  -0.171  23.169  1.00 17.92           C
ATOM   4728  CG  ASN B  92     -19.210   0.960  22.777  1.00 17.67           C
ATOM   4729  OD1 ASN B  92     -18.749   2.066  22.576  1.00 15.73           O
ATOM   4730  ND2 ASN B  92     -20.510   0.686  22.670  1.00 20.42           N
ATOM   4733  C   ASN B  92     -16.081   1.052  22.845  1.00 17.91           C
ATOM   4734  O   ASN B  92     -15.757   1.155  24.021  1.00 17.12           O
ATOM   4735  N   THR B  93     -15.667   1.909  21.902  1.00 17.67           N
ATOM   4737  CA  THR B  93     -14.789   3.068  22.178  1.00 17.77           C
ATOM   4739  CB  THR B  93     -14.457   3.902  20.885  1.00 18.92           C
ATOM   4741  OG1 THR B  93     -13.708   5.097  21.206  1.00 21.52           O
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ATOM   4743  CG2 THR B  93     -15.718   4.434  20.237  1.00 18.95           C
ATOM   4747  C   THR B  93     -15.348   4.006  23.212  1.00 15.15           C
ATOM   4748  O   THR B  93     -14.591   4.719  23.860  1.00 14.57           O
ATOM   4749  N   ASN B  94     -16.676   4.042  23.349  1.00 13.37           N
ATOM   4751  CA  ASN B  94     -17.263   4.874  24.369  1.00 11.96           C
ATOM   4753  CB  ASN B  94     -18.767   5.001  24.180  1.00 12.29           C
ATOM   4756  CG  ASN B  94     -19.122   5.919  23.041  1.00 14.50           C
ATOM   4757  OD1 ASN B  94     -18.348   6.785  22.653  1.00 16.90           O
ATOM   4758  ND2 ASN B  94     -20.312   5.739  22.508  1.00 17.07           N
ATOM   4761  C   ASN B  94     -16.951   4.400  25.772  1.00 10.32           C
ATOM   4762  O   ASN B  94     -17.229   5.130  26.707  1.00 10.75           O
ATOM   4763  N   TYR B  95     -16.361   3.207  25.915  1.00  9.75           N
ATOM   4765  CA  TYR B  95     -15.992   2.654  27.219  1.00  9.39           C
ATOM   4767  CB  TYR B  95     -16.876   1.444  27.541  1.00 10.00           C
ATOM   4770  CG  TYR B  95     -18.334   1.826  27.578  1.00 10.15           C
ATOM   4771  CD1 TYR B  95     -19.127   1.734  26.446  1.00 11.72           C
ATOM   4773  CE1 TYR B  95     -20.466   2.105  26.467  1.00 12.50           C
ATOM   4775  CZ  TYR B  95     -21.008   2.602  27.625  1.00 13.24           C
ATOM   4776  OH  TYR B  95     -22.332   2.984  27.661  1.00 14.96           O
ATOM   4778  CE2 TYR B  95     -20.243   2.720  28.762  1.00 12.98           C
ATOM   4780  CD2 TYR B  95     -18.911   2.333  28.733  1.00 11.90           C
ATOM   4782  C   TYR B  95     -14.512   2.285  27.263  1.00  8.95           C
ATOM   4783  O   TYR B  95     -14.114   1.400  28.010  1.00  8.99           O
ATOM   4784  N   ASP B  96     -13.695   2.996  26.485  1.00  8.73           N
ATOM   4786  CA  ASP B  96     -12.272   2.693  26.401  1.00  8.57           C
ATOM   4788  CB  ASP B  96     -11.716   3.169  25.067  1.00  8.34           C
ATOM   4791  CG  ASP B  96     -10.298   2.720  24.829  1.00  8.28           C
ATOM   4792  OD1 ASP B  96      -9.773   3.069  23.732  1.00  8.57           O
ATOM   4793  OD2 ASP B  96      -9.674   2.040  25.677  1.00  8.50           O
ATOM   4794  C   ASP B  96     -11.510   3.314  27.580  1.00  7.91           C
ATOM   4795  O   ASP B  96     -11.002   4.442  27.510  1.00  8.32           O
ATOM   4796  N   TYR B  97     -11.479   2.567  28.671  1.00  8.03           N
ATOM   4798  CA  TYR B  97     -10.719   2.910  29.860  1.00  7.75           C
ATOM   4800  CB  TYR B  97     -11.386   3.992  30.707  1.00  7.83           C
ATOM   4803  CG  TYR B  97     -12.688   3.607  31.371  1.00  8.16           C
ATOM   4804  CD1 TYR B  97     -13.893   3.674  30.681  1.00  8.43           C
ATOM   4806  CE1 TYR B  97     -15.092   3.350  31.297  1.00  8.51           C
ATOM   4808  CZ  TYR B  97     -15.094   2.959  32.628  1.00  8.68           C
ATOM   4809  OH  TYR B  97     -16.265   2.673  33.298  1.00  9.75           O
ATOM   4811  CE2 TYR B  97     -13.906   2.878  33.321  1.00  8.95           C
ATOM   4813  CD2 TYR B  97     -12.719   3.205  32.697  1.00  8.55           C
ATOM   4815  C   TYR B  97     -10.531   1.625  30.653  1.00  7.47           C
ATOM   4816  O   TYR B  97     -11.168   0.607  30.383  1.00  7.98           O
ATOM   4817  N   GLY B  98      -9.659   1.684  31.647  1.00  7.59           N
ATOM   4819  CA  GLY B  98      -9.409   0.542  32.502  1.00  7.74           C
ATOM   4822  C   GLY B  98      -8.451   0.917  33.603  1.00  7.36           C
ATOM   4823  O   GLY B  98      -8.061   2.082  33.748  1.00  7.93           O
ATOM   4824  N   ALA B  99      -8.079  -0.080  34.390  1.00  7.67           N
ATOM   4826  CA  ALA B  99      -7.139   0.136  35.465  1.00  7.61           C
ATOM   4828  CB  ALA B  99      -7.846   0.428  36.770  1.00  8.42           C
ATOM   4832  C   ALA B  99      -6.207  -1.042  35.626  1.00  8.10           C
ATOM   4833  O   ALA B  99      -6.523  -2.172  35.222  1.00  8.34           O
ATOM   4834  N   ILE B 100      -5.045  -0.762  36.211  1.00  8.17           N
ATOM   4836  CA  ILE B 100      -4.042  -1.770  36.490  1.00  8.36           C
ATOM   4838  CB  ILE B 100      -2.709  -1.485  35.749  1.00  8.61           C
ATOM   4840  CG1 ILE B 100      -2.941  -1.193  34.265  1.00  8.93           C
ATOM   4843  CD1 ILE B 100      -1.682  -0.873  33.485  1.00 10.13           C
ATOM   4847  CG2 ILE B 100      -1.738  -2.640  35.958  1.00  9.23           C
ATOM   4851  C   ILE B 100      -3.764  -1.741  37.982  1.00  8.16           C
ATOM   4852  O   ILE B 100      -3.527  -0.682  38.549  1.00  8.74           O
ATOM   4853  N   GLU B 101      -3.784  -2.903  38.627  1.00  8.55           N
ATOM   4855  CA  GLU B 101      -3.315  -3.015  40.003  1.00  8.71           C
ATOM   4857  CB  GLU B 101      -4.160  -4.001  40.797  1.00  9.18           C
ATOM   4860  CG  GLU B 101      -3.907  -3.943  42.293  1.00 10.20           C
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ATOM   4863  CD  GLU B 101      -5.020  -4.604  43.089  1.00 10.31           C
ATOM   4864  OE1 GLU B 101      -4.713  -5.401  43.998  1.00 12.33           O
ATOM   4865  OE2 GLU B 101      -6.210  -4.354  42.782  1.00 11.03           O
ATOM   4866  C   GLU B 101      -1.858  -3.452  39.989  1.00  8.34           C
ATOM   4867  O   GLU B 101      -1.466  -4.253  39.161  1.00  9.30           O
ATOM   4868  N   LEU B 102      -1.073  -2.887  40.894  1.00  8.98           N
ATOM   4870  CA  LEU B 102       0.358  -3.079  40.934  1.00  8.90           C
ATOM   4872  CB  LEU B 102       1.068  -1.728  41.077  1.00  9.08           C
ATOM   4875  CG  LEU B 102       0.752  -0.722  39.978  1.00 10.14           C
ATOM   4877  CD1 LEU B 102       1.517   0.561  40.225  1.00 10.89           C
ATOM   4881  CD2 LEU B 102       1.034  -1.294  38.585  1.00 10.99           C
ATOM   4885  C   LEU B 102       0.807  -3.976  42.080  1.00  9.48           C
ATOM   4886  O   LEU B 102       0.168  -4.061  43.133  1.00 10.17           O
ATOM   4887  N   SER B 103       1.969  -4.589  41.866  1.00  9.89           N
ATOM   4889  CA  SER B 103       2.601  -5.483  42.828  1.00 10.79           C
ATOM   4891  CB  SER B 103       3.736  -6.273  42.146  1.00 11.99           C
ATOM   4894  OG  SER B 103       4.697  -5.398  41.584  1.00 15.05           O
ATOM   4896  C   SER B 103       3.183  -4.776  44.053  1.00 10.53           C
ATOM   4897  O   SER B 103       3.490  -5.433  45.047  1.00 11.56           O
ATOM   4898  N   GLU B 104       3.367  -3.464  43.962  1.00 10.04           N
ATOM   4900  CA  GLU B 104       3.968  -2.672  45.021  1.00 10.08           C
ATOM   4902  CB  GLU B 104       5.443  -2.395  44.738  1.00 10.68           C
ATOM   4905  CG  GLU B 104       6.259  -3.644  44.449  1.00 11.66           C
ATOM   4908  CD  GLU B 104       7.723  -3.350  44.233  1.00 13.72           C
ATOM   4909  OE1 GLU B 104       8.329  -2.659  45.084  1.00 14.76           O
ATOM   4910  OE2 GLU B 104       8.261  -3.802  43.208  1.00 19.30           O
ATOM   4911  C   GLU B 104       3.227  -1.351  45.093  1.00  9.84           C
ATOM   4912  O   GLU B 104       2.802  -0.809  44.065  1.00 10.32           O
ATOM   4913  N   PRO B 105       3.068  -0.805  46.291  1.00  9.86           N
ATOM   4914  CA  PRO B 105       2.283   0.420  46.478  1.00 10.40           C
ATOM   4916  CB  PRO B 105       1.878   0.324  47.944  1.00 11.33           C
ATOM   4919  CG  PRO B 105       3.053  -0.322  48.587  1.00 11.44           C
ATOM   4922  CD  PRO B 105       3.557  -1.331  47.587  1.00 10.49           C
ATOM   4925  C   PRO B 105       3.075   1.696  46.191  1.00  9.58           C
ATOM   4926  O   PRO B 105       3.227   2.576  47.035  1.00 10.04           O
ATOM   4927  N   ILE B 106       3.538   1.824  44.957  1.00  9.73           N
ATOM   4929  CA  ILE B 106       4.421   2.908  44.586  1.00  9.44           C
ATOM   4931  CB  ILE B 106       5.096   2.600  43.224  1.00  9.89           C
ATOM   4933  CG1 ILE B 106       6.252   3.566  42.933  1.00 10.25           C
ATOM   4936  CD1 ILE B 106       7.381   3.581  43.970  1.00 11.38           C
ATOM   4940  CG2 ILE B 106       4.082   2.599  42.085  1.00 10.23           C
ATOM   4944  C   ILE B 106       3.729   4.271  44.620  1.00  9.14           C
ATOM   4945  O   ILE B 106       4.382   5.305  44.734  1.00  9.49           O
ATOM   4946  N   GLY B 107       2.407   4.287  44.541  1.00  9.13           N
ATOM   4948  CA  GLY B 107       1.648   5.503  44.748  1.00  9.30           C
ATOM   4951  C   GLY B 107       1.833   6.128  46.117  1.00  9.86           C
ATOM   4952  O   GLY B 107       1.627   7.326  46.279  1.00 10.75           O
ATOM   4953  N   ASN B 108       2.228   5.339  47.110  1.00 10.06           N
ATOM   4955  CA  ASN B 108       2.578   5.915  48.400  1.00 11.08           C
ATOM   4957  CB  ASN B 108       2.804   4.831  49.458  1.00 11.79           C
ATOM   4960  CG  ASN B 108       1.518   4.133  49.862  1.00 13.13           C
ATOM   4961  OD1 ASN B 108       0.433   4.675  49.715  1.00 15.54           O
ATOM   4962  ND2 ASN B 108       1.649   2.941  50.428  1.00 15.52           N
ATOM   4965  C   ASN B 108       3.799   6.809  48.340  1.00 11.38           C
ATOM   4966  O   ASN B 108       3.968   7.676  49.192  1.00 13.40           O
ATOM   4967  N   THR B 109       4.644   6.606  47.335  1.00 10.92           N
ATOM   4969  CA  THR B 109       5.811   7.449  47.106  1.00 11.13           C
ATOM   4971  CB  THR B 109       6.961   6.584  46.594  1.00 11.44           C
ATOM   4973  OG1 THR B 109       7.329   5.636  47.604  1.00 13.07           O
ATOM   4975  CG2 THR B 109       8.225   7.390  46.324  1.00 12.36           C
ATOM   4979  C   THR B 109       5.521   8.572  46.123  1.00 10.67           C
ATOM   4980  O   THR B 109       5.856   9.723  46.400  1.00 11.94           O
ATOM   4981  N   VAL B 110       4.931   8.247  44.975  1.00  9.89           N
ATOM   4983  CA  VAL B 110       4.771   9.245  43.921  1.00  9.99           C
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ATOM   4985  CB  VAL B 110       4.883   8.652  42.504  1.00  9.69           C
ATOM   4987  CG1 VAL B 110       6.238   8.008  42.291  1.00 10.32           C
ATOM   4991  CG2 VAL B 110       3.749   7.687  42.194  1.00  9.18           C
ATOM   4995  C   VAL B 110       3.512  10.093  44.054  1.00 10.21           C
ATOM   4996  O   VAL B 110       3.434  11.153  43.425  1.00 11.23           O
ATOM   4997  N   GLY B 111       2.543   9.644  44.840  1.00 10.22           N
ATOM   4999  CA  GLY B 111       1.265  10.314  44.904  1.00 10.24           C
ATOM   5002  C   GLY B 111       0.334   9.866  43.803  1.00  9.73           C
ATOM   5003  O   GLY B 111       0.623   8.938  43.039  1.00 10.12           O
ATOM   5004  N   TYR B 112      -0.815  10.522  43.733  1.00  9.97           N
ATOM   5006  CA  TYR B 112      -1.832  10.140  42.768  1.00  9.95           C
ATOM   5008  CB BTYR B 112      -2.648   8.897  43.221  0.35 10.43           C
ATOM   5009  CB ATYR B 112      -2.598   8.884  43.221  0.65 10.39           C
ATOM   5014  CG BTYR B 112      -2.791   8.641  44.714  0.35 11.58           C
ATOM   5015  CG ATYR B 112      -3.133   8.921  44.615  0.65 11.41           C
ATOM   5016  CD1BTYR B 112      -1.797   7.973  45.428  0.35 12.47           C
ATOM   5017  CD1ATYR B 112      -2.406   8.376  45.672  0.65 13.18           C
ATOM   5020  CE1BTYR B 112      -1.935   7.713  46.789  0.35 13.56           C
ATOM   5021  CE1ATYR B 112      -2.905   8.381  46.970  0.65 15.52           C
ATOM   5024  CZ BTYR B 112      -3.085   8.100  47.449  0.35 14.96           C
ATOM   5025  CZ ATYR B 112      -4.144   8.931  47.209  0.65 16.29           C
ATOM   5026  OH BTYR B 112      -3.215   7.838  48.796  0.35 16.64           O
ATOM   5027  OH ATYR B 112      -4.641   8.940  48.492  0.65 18.51           O
ATOM   5030  CE2BTYR B 112      -4.097   8.743  46.766  0.35 14.31           C
ATOM   5031  CE2ATYR B 112      -4.894   9.467  46.174  0.65 14.98           C
ATOM   5034  CD2BTYR B 112      -3.951   9.007  45.400  0.35 12.89           C
ATOM   5035  CD2ATYR B 112      -4.382   9.459  44.880  0.65 13.02           C
ATOM   5038  C   TYR B 112      -2.745  11.327  42.440  1.00  9.75           C
ATOM   5039  O   TYR B 112      -2.730  12.363  43.110  1.00 10.71           O
ATOM   5040  N   PHE B 113      -3.495  11.159  41.355  1.00  9.55           N
ATOM   5042  CA  PHE B 113      -4.382  12.182  40.822  1.00  9.90           C
ATOM   5044  CB  PHE B 113      -4.592  11.952  39.321  1.00  9.79           C
ATOM   5047  CG  PHE B 113      -3.437  12.384  38.452  1.00  8.75           C
ATOM   5048  CD1 PHE B 113      -3.520  13.562  37.714  1.00  9.30           C
ATOM   5050  CE1 PHE B 113      -2.467  13.968  36.912  1.00 10.00           C
ATOM   5052  CZ  PHE B 113      -1.321  13.222  36.851  1.00  8.88           C
ATOM   5054  CE2 PHE B 113      -1.220  12.060  37.566  1.00  8.70           C
ATOM   5056  CD2 PHE B 113      -2.271  11.633  38.372  1.00  8.97           C
ATOM   5058  C   PHE B 113      -5.772  12.106  41.441  1.00 10.47           C
ATOM   5059  O   PHE B 113      -6.262  11.022  41.775  1.00 11.47           O
ATOM   5060  N   GLY B 114      -6.409  13.267  41.550  1.00 10.79           N
ATOM   5062  CA  GLY B 114      -7.854  13.333  41.663  1.00 11.39           C
ATOM   5065  C   GLY B 114      -8.481  13.125  40.293  1.00 10.67           C
ATOM   5066  O   GLY B 114      -7.801  13.207  39.265  1.00 10.78           O
ATOM   5067  N   TYR B 115      -9.781  12.875  40.278  1.00 10.57           N
ATOM   5069  CA  TYR B 115     -10.524  12.763  39.030  1.00 10.75           C
ATOM   5071  CB  TYR B 115     -10.346  11.382  38.379  1.00 10.71           C
ATOM   5074  CG  TYR B 115     -10.685  10.219  39.275  1.00 10.79           C
ATOM   5075  CD1 TYR B 115     -11.988   9.716  39.338  1.00 10.63           C
ATOM   5077  CE1 TYR B 115     -12.311   8.658  40.183  1.00 10.32           C
ATOM   5079  CZ  TYR B 115     -11.313   8.093  40.968  1.00 10.91           C
ATOM   5080  OH  TYR B 115     -11.581   7.056  41.831  1.00 12.42           O
ATOM   5082  CE2 TYR B 115     -10.021   8.585  40.921  1.00 11.68           C
ATOM   5084  CD2 TYR B 115      -9.715   9.638  40.074  1.00 11.40           C
ATOM   5086  C   TYR B 115     -11.983  13.069  39.319  1.00 10.14           C
ATOM   5087  O   TYR B 115     -12.466  12.866  40.448  1.00 11.11           O
ATOM   5088  N   SER B 116     -12.696  13.556  38.315  1.00 10.15           N
ATOM   5090  CA  SER B 116     -14.058  14.032  38.525  1.00 11.05           C
ATOM   5092  CB  SER B 116     -14.047  15.464  39.061  1.00 12.22           C
ATOM   5095  OG  SER B 116     -15.261  15.741  39.743  1.00 15.63           O
ATOM   5097  C   SER B 116     -14.881  13.963  37.258  1.00 10.80           C
ATOM   5098  O   SER B 116     -14.333  13.881  36.155  1.00 11.34           O
ATOM   5099  N   TYR B 117     -16.198  13.964  37.448  1.00 11.27           N
ATOM   5101  CA  TYR B 117     -17.167  14.054  36.366  1.00 11.28           C
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ATOM   5103  CB  TYR B 117     -18.098  12.834  36.354  1.00 11.24           C
ATOM   5106  CG  TYR B 117     -19.039  12.746  37.533  1.00 11.74           C
ATOM   5107  CD1 TYR B 117     -20.343  13.212  37.431  1.00 13.27           C
ATOM   5109  CE1 TYR B 117     -21.221  13.145  38.509  1.00 15.32           C
ATOM   5111  CZ  TYR B 117     -20.788  12.637  39.708  1.00 15.51           C
ATOM   5112  OH  TYR B 117     -21.659  12.576  40.774  1.00 18.16           O
ATOM   5114  CE2 TYR B 117     -19.494  12.174  39.841  1.00 15.41           C
ATOM   5116  CD2 TYR B 117     -18.626  12.229  38.758  1.00 13.41           C
ATOM   5118  C   TYR B 117     -17.976  15.325  36.528  1.00 12.11           C
ATOM   5119  O   TYR B 117     -18.090  15.880  37.624  1.00 13.00           O
ATOM   5120  N   THR B 118     -18.546  15.790  35.430  1.00 12.32           N
ATOM   5122  CA  THR B 118     -19.471  16.915  35.476  1.00 13.14           C
ATOM   5124  CB BTHR B 118     -18.839  18.242  34.989  0.35 13.58           C
ATOM   5125  CB ATHR B 118     -18.853  18.174  34.815  0.65 13.85           C
ATOM   5128  OG1BTHR B 118     -17.607  18.487  35.674  0.35 14.98           O
ATOM   5129  OG1ATHR B 118     -18.864  18.025  33.391  0.65 12.42           O
ATOM   5132  CG2BTHR B 118     -19.688  19.435  35.421  0.35 13.29           C
ATOM   5133  CG2ATHR B 118     -17.368  18.339  35.127  0.65 14.99           C
ATOM   5140  C   THR B 118     -20.722  16.573  34.714  1.00 13.65           C
ATOM   5141  O   THR B 118     -20.751  15.691  33.870  1.00 14.93           O
ATOM   5142  N   THR B 119     -21.782  17.313  35.018  1.00 14.79           N
ATOM   5144  CA  THR B 119     -23.087  17.127  34.387  1.00 16.50           C
ATOM   5146  CB  THR B 119     -24.192  17.090  35.473  1.00 17.34           C
ATOM   5148  OG1 THR B 119     -24.184  18.319  36.209  1.00 19.76           O
ATOM   5150  CG2 THR B 119     -23.906  16.005  36.521  1.00 18.20           C
ATOM   5154  C   THR B 119     -23.412  18.233  33.389  1.00 16.89           C
ATOM   5155  O   THR B 119     -24.581  18.446  33.065  1.00 18.39           O
ATOM   5156  N   SER B 120     -22.392  18.945  32.932  1.00 15.85           N
ATOM   5158  CA  SER B 120     -22.568  20.023  31.976  1.00 15.52           C
ATOM   5160  CB  SER B 120     -22.688  21.348  32.714  1.00 17.29           C
ATOM   5163  OG  SER B 120     -21.566  21.555  33.538  1.00 19.16           O
ATOM   5165  C   SER B 120     -21.385  20.044  31.015  1.00 13.69           C
ATOM   5166  O   SER B 120     -20.433  19.256  31.151  1.00 13.49           O
ATOM   5167  N   SER B 121     -21.450  20.938  30.037  1.00 13.50           N
ATOM   5169  CA  SER B 121     -20.440  20.977  28.999  1.00 13.20           C
ATOM   5171  CB  SER B 121     -20.823  22.004  27.943  1.00 13.58           C
ATOM   5174  OG  SER B 121     -19.822  22.072  26.951  1.00 14.79           O
ATOM   5176  C   SER B 121     -19.065  21.321  29.561  1.00 12.29           C
ATOM   5177  O   SER B 121     -18.936  22.162  30.445  1.00 13.82           O
ATOM   5178  N   LEU B 122     -18.034  20.659  29.042  1.00 11.06           N
ATOM   5180  CA  LEU B 122     -16.653  20.994  29.362  1.00 10.63           C
ATOM   5182  CB  LEU B 122     -15.870  19.715  29.679  1.00 10.33           C
ATOM   5185  CG  LEU B 122     -16.152  19.154  31.076  1.00 11.02           C
ATOM   5187  CD1 LEU B 122     -15.645  17.729  31.205  1.00 11.81           C
ATOM   5191  CD2 LEU B 122     -15.557  20.038  32.139  1.00 12.56           C
ATOM   5195  C   LEU B 122     -15.968  21.775  28.238  1.00 10.13           C
ATOM   5196  O   LEU B 122     -14.775  22.042  28.324  1.00 10.57           O
ATOM   5197  N   VAL B 123     -16.708  22.183  27.209  1.00 10.64           N
ATOM   5199  CA  VAL B 123     -16.101  22.935  26.115  1.00 10.83           C
ATOM   5201  CB  VAL B 123     -17.123  23.312  25.017  1.00 11.49           C
ATOM   5203  CG1 VAL B 123     -16.511  24.290  24.006  1.00 12.60           C
ATOM   5207  CG2 VAL B 123     -17.603  22.060  24.288  1.00 12.49           C
ATOM   5211  C   VAL B 123     -15.439  24.192  26.669  1.00 10.32           C
ATOM   5212  O   VAL B 123     -16.057  24.936  27.431  1.00 11.74           O
ATOM   5213  N   GLY B 124     -14.189  24.416  26.283  1.00 10.03           N
ATOM   5215  CA  GLY B 124     -13.431  25.575  26.714  1.00 10.35           C
ATOM   5218  C   GLY B 124     -12.591  25.362  27.954  1.00  9.90           C
ATOM   5219  O   GLY B 124     -11.707  26.170  28.220  1.00 11.28           O
ATOM   5220  N   THR B 125     -12.851  24.311  28.726  1.00  9.67           N
ATOM   5222  CA  THR B 125     -12.062  24.049  29.919  1.00  9.49           C
ATOM   5224  CB  THR B 125     -12.709  22.900  30.695  1.00 10.53           C
ATOM   5226  OG1 THR B 125     -13.998  23.310  31.178  1.00 13.20           O
ATOM   5228  CG2 THR B 125     -11.907  22.498  31.922  1.00 10.73           C
ATOM   5232  C   THR B 125     -10.635  23.689  29.511  1.00  8.89           C
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ATOM   5233  O   THR B 125     -10.437  22.872  28.619  1.00  9.49           O
ATOM   5234  N   THR B 126      -9.646  24.285  30.170  1.00  9.07           N
ATOM   5236  CA  THR B 126      -8.254  23.992  29.867  1.00  9.38           C
ATOM   5238  CB  THR B 126      -7.368  25.212  30.064  1.00 10.46           C
ATOM   5240  OG1 THR B 126      -7.532  25.706  31.393  1.00 12.73           O
ATOM   5242  CG2 THR B 126      -7.790  26.346  29.130  1.00 11.44           C
ATOM   5246  C   THR B 126      -7.731  22.819  30.679  1.00  8.61           C
ATOM   5247  O   THR B 126      -8.035  22.654  31.874  1.00  9.84           O
ATOM   5248  N   VAL B 127      -6.951  21.996  29.987  1.00  8.18           N
ATOM   5250  CA  VAL B 127      -6.403  20.764  30.520  1.00  8.03           C
ATOM   5252  CB  VAL B 127      -7.290  19.529  30.187  1.00  8.26           C
ATOM   5254  CG1 VAL B 127      -8.635  19.599  30.912  1.00  9.66           C
ATOM   5258  CG2 VAL B 127      -7.486  19.389  28.694  1.00  9.04           C
ATOM   5262  C   VAL B 127      -5.001  20.543  29.961  1.00  7.96           C
ATOM   5263  O   VAL B 127      -4.625  21.117  28.935  1.00  9.12           O
ATOM   5264  N   THR B 128      -4.259  19.675  30.630  1.00  7.81           N
ATOM   5266  CA  THR B 128      -2.953  19.208  30.209  1.00  7.75           C
ATOM   5268  CB  THR B 128      -1.953  19.374  31.362  1.00  8.07           C
ATOM   5270  OG1 THR B 128      -1.843  20.762  31.705  1.00  9.24           O
ATOM   5272  CG2 THR B 128      -0.549  18.864  31.006  1.00  8.88           C
ATOM   5276  C   THR B 128      -3.052  17.735  29.857  1.00  7.12           C
ATOM   5277  O   THR B 128      -3.715  16.967  30.556  1.00  7.80           O
ATOM   5278  N   ILE B 129      -2.385  17.340  28.775  1.00  6.77           N
ATOM   5280  CA  ILE B 129      -2.233  15.940  28.421  1.00  6.79           C
ATOM   5282  CB  ILE B 129      -2.874  15.613  27.062  1.00  7.12           C
ATOM   5284  CG1 ILE B 129      -4.328  16.098  27.046  1.00  7.95           C
ATOM   5287  CD1 ILE B 129      -5.076  15.825  25.764  1.00  8.74           C
ATOM   5291  CG2 ILE B 129      -2.766  14.141  26.789  1.00  8.17           C
ATOM   5295  C   ILE B 129      -0.739  15.639  28.412  1.00  6.77           C
ATOM   5296  O   ILE B 129       0.001  16.217  27.603  1.00  7.27           O
ATOM   5297  N   SER B 130      -0.298  14.761  29.305  1.00  6.76           N
ATOM   5299  CA  SER B 130       1.112  14.417  29.438  1.00  6.68           C
ATOM   5301  CB  SER B 130       1.694  15.022  30.710  1.00  7.29           C
ATOM   5304  OG  SER B 130       3.097  14.906  30.734  1.00  8.01           O
ATOM   5306  C   SER B 130       1.250  12.911  29.453  1.00  6.73           C
ATOM   5307  O   SER B 130       0.517  12.224  30.158  1.00  6.79           O
ATOM   5308  N   GLY B 131       2.187  12.390  28.665  1.00  6.62           N
ATOM   5310  CA  GLY B 131       2.425  10.958  28.637  1.00  6.73           C
ATOM   5313  C   GLY B 131       3.640  10.604  27.804  1.00  6.56           C
ATOM   5314  O   GLY B 131       4.554  11.409  27.661  1.00  7.16           O
ATOM   5315  N   TYR B 132       3.652   9.381  27.288  1.00  6.84           N
ATOM   5317  CA  TYR B 132       4.858   8.737  26.740  1.00  7.12           C
ATOM   5319  CB  TYR B 132       5.165   7.463  27.555  1.00  7.14           C
ATOM   5322  CG  TYR B 132       5.728   7.832  28.917  1.00  7.14           C
ATOM   5323  CD1 TYR B 132       7.087   8.103  29.060  1.00  7.37           C
ATOM   5325  CE1 TYR B 132       7.614   8.520  30.265  1.00  7.89           C
ATOM   5327  CZ  TYR B 132       6.781   8.669  31.364  1.00  7.57           C
ATOM   5328  OH  TYR B 132       7.269   9.112  32.573  1.00  8.04           O
ATOM   5330  CE2 TYR B 132       5.438   8.389  31.262  1.00  7.67           C
ATOM   5332  CD2 TYR B 132       4.908   7.980  30.035  1.00  7.29           C
ATOM   5334  C   TYR B 132       4.676   8.424  25.250  1.00  6.94           C
ATOM   5335  O   TYR B 132       4.361   7.295  24.880  1.00  8.05           O
ATOM   5336  N   PRO B 133       4.874   9.411  24.378  1.00  7.25           N
ATOM   5337  CA  PRO B 133       4.670   9.185  22.944  1.00  7.42           C
ATOM   5339  CB  PRO B 133       4.628  10.594  22.368  1.00  8.13           C
ATOM   5342  CG  PRO B 133       5.503  11.387  23.285  1.00  8.21           C
ATOM   5345  CD  PRO B 133       5.210  10.826  24.655  1.00  7.47           C
ATOM   5348  C   PRO B 133       5.786   8.400  22.267  1.00  7.76           C
ATOM   5349  O   PRO B 133       6.974   8.597  22.533  1.00  8.78           O
ATOM   5350  N   GLY B 134       5.389   7.581  21.300  1.00  8.21           N
ATOM   5352  CA  GLY B 134       6.306   6.749  20.548  1.00  9.26           C
ATOM   5355  C   GLY B 134       7.046   7.440  19.418  1.00  9.69           C
ATOM   5356  O   GLY B 134       7.926   6.828  18.819  1.00 12.46           O
ATOM   5357  N   ASP B 135       6.718   8.697  19.134  1.00  8.82           N
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ATOM   5359  CA  ASP B 135       7.459   9.489  18.154  1.00  9.23           C
ATOM   5361  CB  ASP B 135       6.533  10.305  17.243  1.00  8.88           C
ATOM   5364  CG  ASP B 135       5.732  11.364  17.966  1.00  8.72           C
ATOM   5365  OD1 ASP B 135       5.506  11.238  19.200  1.00  8.57           O
ATOM   5366  OD2 ASP B 135       5.290  12.341  17.292  1.00  9.32           O
ATOM   5367  C   ASP B 135       8.523  10.368  18.796  1.00  9.65           C
ATOM   5368  O   ASP B 135       9.102  11.216  18.121  1.00 11.42           O
ATOM   5369  N   LYS B 136       8.768  10.161  20.088  1.00  9.77           N
ATOM   5371  CA  LYS B 136       9.873  10.781  20.812  1.00 10.15           C
ATOM   5373  CB  LYS B 136       9.349  11.647  21.958  1.00  9.99           C
ATOM   5376  CG  LYS B 136       8.378  12.734  21.523  1.00 10.04           C
ATOM   5379  CD  LYS B 136       9.008  13.792  20.637  1.00 11.86           C
ATOM   5382  CE  LYS B 136       8.014  14.925  20.392  1.00 13.11           C
ATOM   5385  NZ  LYS B 136       8.453  15.910  19.384  1.00 15.13           N
ATOM   5389  C   LYS B 136      10.756   9.670  21.376  1.00 10.43           C
ATOM   5390  O   LYS B 136      10.432   8.491  21.280  1.00 11.37           O
ATOM   5391  N   THR B 137      11.881  10.042  21.976  1.00 11.03           N
ATOM   5393  CA  THR B 137      12.777   9.068  22.582  1.00 11.49           C
ATOM   5395  CB  THR B 137      13.887   9.813  23.343  1.00 12.46           C
ATOM   5397  OG1 THR B 137      14.687  10.558  22.415  1.00 14.16           O
ATOM   5399  CG2 THR B 137      14.865   8.837  24.040  1.00 13.85           C
ATOM   5403  C   THR B 137      12.010   8.169  23.536  1.00 10.34           C
ATOM   5404  O   THR B 137      11.257   8.654  24.378  1.00  9.84           O
ATOM   5405  N   ALA B 138      12.240   6.868  23.428  1.00 10.82           N
ATOM   5407  CA  ALA B 138      11.524   5.900  24.232  1.00 10.91           C
ATOM   5409  CB  ALA B 138      12.055   4.511  23.995  1.00 11.85           C
ATOM   5413  C   ALA B 138      11.631   6.256  25.702  1.00 10.45           C
ATOM   5414  O   ALA B 138      12.694   6.552  26.218  1.00 11.32           O
ATOM   5415  N   GLY B 139      10.503   6.194  26.378  1.00 10.16           N
ATOM   5417  CA  GLY B 139      10.468   6.419  27.800  1.00  9.75           C
ATOM   5420  C   GLY B 139      10.535   7.861  28.250  1.00  8.52           C
ATOM   5421  O   GLY B 139      10.669   8.089  29.441  1.00  9.08           O
ATOM   5422  N   THR B 140      10.421   8.829  27.340  1.00  8.37           N
ATOM   5424  CA  THR B 140      10.416  10.238  27.729  1.00  8.32           C
ATOM   5426  CB  THR B 140      11.318  11.119  26.843  1.00  9.11           C
ATOM   5428  OG1 THR B 140      10.877  11.095  25.476  1.00  9.56           O
ATOM   5430  CG2 THR B 140      12.768  10.611  26.900  1.00 10.40           C
ATOM   5434  C   THR B 140       8.987  10.774  27.783  1.00  7.64           C
ATOM   5435  O   THR B 140       8.120  10.378  26.991  1.00  8.11           O
ATOM   5436  N   GLN B 141       8.736  11.644  28.753  1.00  7.31           N
ATOM   5438  CA  GLN B 141       7.405  12.176  28.997  1.00  7.20           C
ATOM   5440  CB  GLN B 141       7.108  12.226  30.513  1.00  7.37           C
ATOM   5443  CG  GLN B 141       5.617  12.242  30.802  1.00  7.74           C
ATOM   5446  CD  GLN B 141       5.238  12.460  32.256  1.00  7.09           C
ATOM   5447  OE1 GLN B 141       4.394  13.318  32.560  1.00  8.12           O
ATOM   5448  NE2 GLN B 141       5.812  11.669  33.171  1.00  7.80           N
ATOM   5451  C   GLN B 141       7.284  13.551  28.353  1.00  7.04           C
ATOM   5452  O   GLN B 141       8.177  14.384  28.523  1.00  7.62           O
ATOM   5453  N   TRP B 142       6.180  13.771  27.652  1.00  7.07           N
ATOM   5455  CA  TRP B 142       5.912  14.982  26.902  1.00  7.12           C
ATOM   5457  CB  TRP B 142       6.063  14.717  25.387  1.00  7.52           C
ATOM   5460  CG  TRP B 142       7.481  14.449  24.987  1.00  7.95           C
ATOM   5461  CD1 TRP B 142       8.205  13.342  25.263  1.00  7.71           C
ATOM   5463  NE1 TRP B 142       9.486  13.476  24.796  1.00  8.83           N
ATOM   5465  CE2 TRP B 142       9.612  14.702  24.206  1.00  8.82           C
ATOM   5466  CD2 TRP B 142       8.363  15.342  24.303  1.00  8.41           C
ATOM   5467  CE3 TRP B 142       8.227  16.617  23.758  1.00  9.13           C
ATOM   5469  CZ3 TRP B 142       9.321  17.200  23.122  1.00  9.87           C
ATOM   5471  CH2 TRP B 142      10.538  16.541  23.048  1.00 10.47           C
ATOM   5473  CZ2 TRP B 142      10.703  15.288  23.568  1.00 10.08           C
ATOM   5475  C   TRP B 142       4.492  15.441  27.166  1.00  6.82           C
ATOM   5476  O   TRP B 142       3.594  14.623  27.352  1.00  7.54           O
ATOM   5477  N   GLN B 143       4.282  16.757  27.146  1.00  6.91           N
ATOM   5479  CA  GLN B 143       2.998  17.337  27.499  1.00  6.91           C
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ATOM   5481  CB  GLN B 143       3.012  17.856  28.938  1.00  7.31           C
ATOM   5484  CG  GLN B 143       3.928  19.058  29.162  1.00  8.18           C
ATOM   5487  CD  GLN B 143       3.867  19.564  30.570  1.00  8.88           C
ATOM   5488  OE1 GLN B 143       2.792  19.555  31.173  1.00 10.18           O
ATOM   5489  NE2 GLN B 143       4.988  20.039  31.087  1.00 10.86           N
ATOM   5492  C   GLN B 143       2.599  18.460  26.561  1.00  7.08           C
ATOM   5493  O   GLN B 143       3.427  19.104  25.928  1.00  7.42           O
ATOM   5494  N   HIS B 144       1.296  18.711  26.521  1.00  7.02           N
ATOM   5496  CA  HIS B 144       0.706  19.829  25.804  1.00  7.09           C
ATOM   5498  CB  HIS B 144       0.457  19.463  24.342  1.00  7.49           C
ATOM   5501  CG  HIS B 144       0.061  20.617  23.491  1.00  7.31           C
ATOM   5502  ND1 HIS B 144      -0.682  20.454  22.350  1.00  8.42           N
ATOM   5504  CE1 HIS B 144      -0.861  21.643  21.800  1.00  8.61           C
ATOM   5506  NE2 HIS B 144      -0.286  22.564  22.557  1.00  8.06           N
ATOM   5508  CD2 HIS B 144       0.319  21.942  23.610  1.00  8.23           C
ATOM   5510  C   HIS B 144      -0.604  20.173  26.496  1.00  6.98           C
ATOM   5511  O   HIS B 144      -1.362  19.276  26.890  1.00  8.41           O
ATOM   5512  N   SER B 145      -0.878  21.463  26.640  1.00  7.81           N
ATOM   5514  CA  SER B 145      -2.093  21.946  27.290  1.00  7.65           C
ATOM   5516  CB  SER B 145      -1.755  22.780  28.522  1.00  8.72           C
ATOM   5519  OG  SER B 145      -1.027  22.028  29.472  1.00  9.99           O
ATOM   5521  C   SER B 145      -2.927  22.764  26.315  1.00  7.50           C
ATOM   5522  O   SER B 145      -2.406  23.304  25.338  1.00  8.51           O
ATOM   5523  N   GLY B 146      -4.218  22.863  26.598  1.00  7.67           N
ATOM   5525  CA  GLY B 146      -5.132  23.637  25.793  1.00  7.84           C
ATOM   5528  C   GLY B 146      -6.563  23.318  26.170  1.00  7.50           C
ATOM   5529  O   GLY B 146      -6.830  22.629  27.148  1.00  8.13           O
ATOM   5530  N   PRO B 147      -7.503  23.835  25.402  1.00  7.99           N
ATOM   5531  CA  PRO B 147      -8.924  23.707  25.733  1.00  8.42           C
ATOM   5533  CB  PRO B 147      -9.524  24.935  25.053  1.00  8.99           C
ATOM   5536  CG  PRO B 147      -8.686  25.096  23.804  1.00  9.17           C
ATOM   5539  CD  PRO B 147      -7.290  24.658  24.195  1.00  8.66           C
ATOM   5542  C   PRO B 147      -9.581  22.445  25.182  1.00  8.24           C
ATOM   5543  O   PRO B 147      -9.216  21.930  24.128  1.00  8.60           O
ATOM   5544  N   ILE B 148     -10.613  21.991  25.892  1.00  8.18           N
ATOM   5546  CA  ILE B 148     -11.532  21.003  25.349  1.00  8.21           C
ATOM   5548  CB  ILE B 148     -12.458  20.481  26.455  1.00  8.12           C
ATOM   5550  CG1 ILE B 148     -11.654  19.795  27.570  1.00  9.56           C
ATOM   5553  CD1 ILE B 148     -10.843  18.627  27.145  1.00 10.59           C
ATOM   5557  CG2 ILE B 148     -13.529  19.585  25.887  1.00  8.52           C
ATOM   5561  C   ILE B 148     -12.338  21.662  24.222  1.00  8.21           C
ATOM   5562  O   ILE B 148     -12.939  22.728  24.410  1.00  9.55           O
ATOM   5563  N   ALA B 149     -12.348  21.019  23.064  1.00  8.67           N
ATOM   5565  CA  ALA B 149     -13.055  21.532  21.896  1.00  9.10           C
ATOM   5567  CB  ALA B 149     -12.286  21.197  20.632  1.00  9.84           C
ATOM   5571  C   ALA B 149     -14.476  21.013  21.771  1.00  9.41           C
ATOM   5572  O   ALA B 149     -15.352  21.743  21.301  1.00 10.63           O
ATOM   5573  N   ILE B 150     -14.684  19.747  22.136  1.00  9.20           N
ATOM   5575  CA  ILE B 150     -15.983  19.098  22.036  1.00 10.09           C
ATOM   5577  CB  ILE B 150     -16.093  18.145  20.814  1.00 10.95           C
ATOM   5579  CG1 ILE B 150     -15.739  18.858  19.510  1.00 10.69           C
ATOM   5582  CD1 ILE B 150     -15.768  17.974  18.271  1.00 11.51           C
ATOM   5586  CG2 ILE B 150     -17.497  17.568  20.704  1.00 13.68           C
ATOM   5590  C   ILE B 150     -16.183  18.306  23.320  1.00  9.44           C
ATOM   5591  O   ILE B 150     -15.291  17.594  23.769  1.00  8.76           O
ATOM   5592  N   SER B 151     -17.372  18.427  23.889  1.00 10.89           N
ATOM   5594  CA  SER B 151     -17.765  17.741  25.101  1.00 11.77           C
ATOM   5596  CB  SER B 151     -18.167  18.810  26.136  1.00 12.97           C
ATOM   5599  OG  SER B 151     -18.512  18.242  27.381  1.00 15.09           O
ATOM   5601  C   SER B 151     -18.973  16.866  24.767  1.00 11.59           C
ATOM   5602  O   SER B 151     -20.087  17.374  24.707  1.00 14.34           O
ATOM   5603  N   GLU B 152     -18.761  15.581  24.505  1.00 11.06           N
ATOM   5605  CA  GLU B 152     -19.854  14.663  24.213  1.00 10.89           C
ATOM   5607  CB BGLU B 152     -19.631  13.897  22.888  0.35 11.93           C
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ATOM   5608  CB AGLU B 152     -19.474  13.796  23.024  0.65 12.76           C
ATOM   5613  CG BGLU B 152     -20.041  14.715  21.652  0.35 12.04           C
ATOM   5614  CG AGLU B 152     -18.748  14.582  21.953  0.65 14.68           C
ATOM   5619  CD BGLU B 152     -20.198  13.882  20.388  0.35 13.76           C
ATOM   5620  CD AGLU B 152     -18.197  13.685  20.889  0.65 17.47           C
ATOM   5621  OE1BGLU B 152     -21.155  14.126  19.613  0.35 15.60           O
ATOM   5622  OE1AGLU B 152     -18.974  13.391  19.960  0.65 19.10           O
ATOM   5623  OE2BGLU B 152     -19.369  12.976  20.169  0.35 15.01           O
ATOM   5624  OE2AGLU B 152     -17.012  13.276  20.998  0.65 18.63           O
ATOM   5625  C    GLU B 152     -20.076  13.771  25.417  1.00 10.28           C
ATOM   5626  O    GLU B 152     -19.376  13.873  26.426  1.00 11.20           O
ATOM   5627  N    THR B 153     -21.057  12.893  25.338  1.00  9.81           N
ATOM   5629  CA   THR B 153     -21.430  12.101  26.492  1.00 10.13           C
ATOM   5631  CB   THR B 153     -22.622  11.232  26.129  1.00 10.71           C
ATOM   5633  OG1  THR B 153     -23.706  12.086  25.751  1.00 12.66           O
ATOM   5635  CG2  THR B 153     -23.106  10.417  27.332  1.00 11.52           C
ATOM   5639  C    THR B 153     -20.286  11.246  27.012  1.00  9.76           C
ATOM   5640  O    THR B 153     -20.065  11.197  28.214  1.00 10.41           O
ATOM   5641  N    TYR B 154     -19.588  10.574  26.108  1.00  9.41           N
ATOM   5643  CA   TYR B 154     -18.588   9.578  26.489  1.00  9.25           C
ATOM   5645  CB   TYR B 154     -18.890   8.217  25.847  1.00  9.78           C
ATOM   5648  CG   TYR B 154     -20.239   7.693  26.220  1.00 10.14           C
ATOM   5649  CD1  TYR B 154     -20.470   7.150  27.475  1.00 10.50           C
ATOM   5651  CE1  TYR B 154     -21.712   6.673  27.838  1.00 10.83           C
ATOM   5653  CZ   TYR B 154     -22.755   6.754  26.930  1.00 11.01           C
ATOM   5654  OH   TYR B 154     -24.016   6.299  27.224  1.00 12.69           O
ATOM   5656  CE2  TYR B 154     -22.535   7.282  25.686  1.00 11.61           C
ATOM   5658  CD2  TYR B 154     -21.293   7.762  25.344  1.00 11.24           C
ATOM   5660  C    TYR B 154     -17.172   9.972  26.116  1.00  8.89           C
ATOM   5661  O    TYR B 154     -16.233   9.282  26.532  1.00  9.04           O
ATOM   5662  N    LYS B 155     -17.001  11.045  25.351  1.00  9.30           N
ATOM   5664  CA   LYS B 155     -15.694  11.445  24.860  1.00  9.53           C
ATOM   5666  CB BLYS B 155     -15.479  11.009  23.401  0.35 10.42           C
ATOM   5667  CB ALYS B 155     -15.521  11.057  23.393  0.65 10.60           C
ATOM   5672  CG BLYS B 155     -15.714   9.526  23.080  0.35 11.44           C
ATOM   5673  CG ALYS B 155     -15.446   9.579  23.102  0.65 11.66           C
ATOM   5678  CD BLYS B 155     -14.796   8.573  23.861  0.35 11.42           C
ATOM   5679  CD ALYS B 155     -14.096   8.991  23.466  0.65  9.69           C
ATOM   5684  CE BLYS B 155     -13.424   8.327  23.221  0.35 11.05           C
ATOM   5685  CE ALYS B 155     -14.129   7.489  23.408  0.65 12.11           C
ATOM   5690  NZ BLYS B 155     -12.677   7.235  23.943  0.35 10.75           N
ATOM   5691  NZ ALYS B 155     -12.784   6.834  23.478  0.65 10.75           N
ATOM   5698  C    LYS B 155     -15.565  12.944  24.954  1.00  9.70           C
ATOM   5699  O    LYS B 155     -16.531  13.686  24.765  1.00 11.39           O
ATOM   5700  N    LEU B 156     -14.365  13.388  25.280  1.00  8.75           N
ATOM   5702  CA   LEU B 156     -13.957  14.757  25.042  1.00  8.77           C
ATOM   5704  CB   LEU B 156     -13.188  15.313  26.238  1.00  9.34           C
ATOM   5707  CG   LEU B 156     -13.899  15.230  27.589  1.00  9.50           C
ATOM   5709  CD1  LEU B 156     -13.075  15.921  28.641  1.00 10.29           C
ATOM   5713  CD2  LEU B 156     -15.313  15.818  27.545  1.00 10.26           C
ATOM   5717  C    LEU B 156     -13.063  14.781  23.817  1.00  8.24           C
ATOM   5718  O    LEU B 156     -12.322  13.817  23.555  1.00  9.48           O
ATOM   5719  N    GLN B 157     -13.115  15.863  23.049  1.00  7.55           N
ATOM   5721  CA   GLN B 157     -12.210  16.040  21.931  1.00  7.62           C
ATOM   5723  CB   GLN B 157     -12.926  15.991  20.589  1.00  8.06           C
ATOM   5726  CG   GLN B 157     -13.830  14.779  20.448  1.00  8.73           C
ATOM   5729  CD   GLN B 157     -14.089  14.415  19.009  1.00  9.08           C
ATOM   5730  OE1  GLN B 157     -13.254  14.641  18.152  1.00 11.00           O
ATOM   5731  NE2  GLN B 157     -15.236  13.811  18.749  1.00 10.76           N
ATOM   5734  C    GLN B 157     -11.462  17.344  22.077  1.00  7.12           C
ATOM   5735  O    GLN B 157     -11.942  18.287  22.701  1.00  7.80           O
ATOM   5736  N    TYR B 158     -10.267  17.376  21.508  1.00  7.08           N
ATOM   5738  CA   TYR B 158      -9.332  18.471  21.731  1.00  7.13           C
ATOM   5740  CB   TYR B 158      -8.677  18.365  23.128  1.00  6.96           C
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ATOM   5743  CG  TYR B 158      -8.393  16.941  23.559  1.00  7.06           C
ATOM   5744  CD1 TYR B 158      -7.326  16.222  23.034  1.00  7.19           C
ATOM   5746  CE1 TYR B 158      -7.108  14.901  23.404  1.00  7.09           C
ATOM   5748  CZ  TYR B 158      -7.946  14.281  24.320  1.00  7.18           C
ATOM   5749  OH  TYR B 158      -7.750  12.979  24.733  1.00  7.90           O
ATOM   5751  CE2 TYR B 158      -9.012  14.992  24.839  1.00  7.53           C
ATOM   5753  CD2 TYR B 158      -9.232  16.292  24.455  1.00  7.22           C
ATOM   5755  C   TYR B 158      -8.280  18.442  20.641  1.00  6.81           C
ATOM   5756  O   TYR B 158      -8.009  17.396  20.043  1.00  7.32           O
ATOM   5757  N   ALA B 159      -7.670  19.601  20.393  1.00  7.32           N
ATOM   5759  CA  ALA B 159      -6.629  19.703  19.374  1.00  7.78           C
ATOM   5761  CB  ALA B 159      -6.680  21.052  18.663  1.00  8.55           C
ATOM   5765  C   ALA B 159      -5.229  19.451  19.903  1.00  7.90           C
ATOM   5766  O   ALA B 159      -4.297  19.384  19.110  1.00  8.83           O
ATOM   5767  N   MET B 160      -5.055  19.302  21.220  1.00  7.36           N
ATOM   5769  CA  MET B 160      -3.713  19.139  21.772  1.00  7.42           C
ATOM   5771  CB  MET B 160      -3.733  18.986  23.293  1.00  8.13           C
ATOM   5774  CG  MET B 160      -4.060  20.269  24.029  1.00  8.32           C
ATOM   5777  SD  MET B 160      -5.812  20.754  24.003  1.00  8.06           S
ATOM   5778  CE  MET B 160      -6.418  19.818  25.409  1.00  8.63           C
ATOM   5782  C   MET B 160      -3.042  17.927  21.119  1.00  7.49           C
ATOM   5783  O   MET B 160      -3.660  16.882  20.868  1.00  7.73           O
ATOM   5784  N   ASP B 161      -1.756  18.098  20.866  1.00  7.41           N
ATOM   5786  CA  ASP B 161      -0.986  17.115  20.130  1.00  7.72           C
ATOM   5788  CB  ASP B 161       0.316  17.758  19.654  1.00  8.40           C
ATOM   5791  CG  ASP B 161       0.065  18.961  18.781  1.00  8.51           C
ATOM   5792  OD1 ASP B 161       0.577  20.072  19.078  1.00  9.92           O
ATOM   5793  OD2 ASP B 161      -0.668  18.829  17.794  1.00  8.73           O
ATOM   5794  C   ASP B 161      -0.704  15.870  20.953  1.00  7.16           C
ATOM   5795  O   ASP B 161      -0.294  15.963  22.117  1.00  7.81           O
ATOM   5796  N   THR B 162      -0.901  14.722  20.319  1.00  7.20           N
ATOM   5798  CA  THR B 162      -0.669  13.420  20.924  1.00  7.17           C
ATOM   5800  CB  THR B 162      -1.969  12.811  21.499  1.00  7.29           C
ATOM   5802  OG1 THR B 162      -2.905  12.578  20.436  1.00  7.90           O
ATOM   5804  CG2 THR B 162      -2.645  13.727  22.509  1.00  8.12           C
ATOM   5808  C   THR B 162      -0.154  12.465  19.857  1.00  7.27           C
ATOM   5809  O   THR B 162      -0.332  12.693  18.664  1.00  7.57           O
ATOM   5810  N   TYR B 163       0.414  11.350  20.298  1.00  7.74           N
ATOM   5812  CA  TYR B 163       0.840  10.282  19.401  1.00  7.85           C
ATOM   5814  CB  TYR B 163       2.316  10.465  19.013  1.00  8.00           C
ATOM   5817  CG  TYR B 163       2.766   9.721  17.771  1.00  8.39           C
ATOM   5818  CD1 TYR B 163       2.621  10.309  16.533  1.00 10.58           C
ATOM   5820  CE1 TYR B 163       3.039   9.684  15.385  1.00 11.78           C
ATOM   5822  CZ  TYR B 163       3.642   8.452  15.458  1.00 11.29           C
ATOM   5823  OH  TYR B 163       4.037   7.861  14.280  1.00 14.10           O
ATOM   5825  CE2 TYR B 163       3.807   7.835  16.689  1.00  9.46           C
ATOM   5827  CD2 TYR B 163       3.390   8.484  17.833  1.00  8.33           C
ATOM   5829  C   TYR B 163       0.643   8.948  20.088  1.00  7.90           C
ATOM   5830  O   TYR B 163       0.537   8.870  21.310  1.00  8.15           O
ATOM   5831  N   GLY B 164       0.628   7.881  19.296  1.00  7.76           N
ATOM   5833  CA  GLY B 164       0.677   6.530  19.828  1.00  8.11           C
ATOM   5836  C   GLY B 164       1.667   6.417  20.964  1.00  7.49           C
ATOM   5837  O   GLY B 164       2.773   6.926  20.880  1.00  8.72           O
ATOM   5838  N   GLY B 165       1.262   5.708  22.009  1.00  7.45           N
ATOM   5840  CA  GLY B 165       1.988   5.644  23.269  1.00  7.27           C
ATOM   5843  C   GLY B 165       1.350   6.507  24.339  1.00  6.66           C
ATOM   5844  O   GLY B 165       1.461   6.214  25.531  1.00  7.23           O
ATOM   5845  N   GLN B 166       0.662   7.572  23.923  1.00  6.83           N
ATOM   5847  CA  GLN B 166      -0.003   8.463  24.859  1.00  6.61           C
ATOM   5849  CB  GLN B 166       0.045   9.919  24.381  1.00  6.74           C
ATOM   5852  CG  GLN B 166       1.451  10.489  24.459  1.00  7.33           C
ATOM   5855  CD  GLN B 166       1.507  11.943  24.074  1.00  6.80           C
ATOM   5856  OE1 GLN B 166       1.609  12.277  22.895  1.00  7.44           O
ATOM   5857  NE2 GLN B 166       1.421  12.831  25.056  1.00  8.44           N
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ATOM   5860  C   GLN B 166      -1.429   8.054  25.211  1.00  6.58           C
ATOM   5861  O   GLN B 166      -2.003   8.683  26.096  1.00  6.83           O
ATOM   5862  N   ALA B 167      -2.023   7.043  24.587  1.00  6.87           N
ATOM   5864  CA  ALA B 167      -3.285   6.550  25.133  1.00  6.77           C
ATOM   5866  CB  ALA B 167      -3.854   5.379  24.386  1.00  7.55           C
ATOM   5870  C   ALA B 167      -3.038   6.162  26.587  1.00  6.83           C
ATOM   5871  O   ALA B 167      -1.998   5.619  26.939  1.00  7.16           O
ATOM   5872  N   GLY B 168      -4.029   6.461  27.410  1.00  6.56           N
ATOM   5874  CA  GLY B 168      -3.940   6.275  28.838  1.00  6.85           C
ATOM   5877  C   GLY B 168      -3.482   7.506  29.584  1.00  6.95           C
ATOM   5878  O   GLY B 168      -3.573   7.528  30.811  1.00  8.08           O
ATOM   5879  N   SER B 169      -2.983   8.524  28.883  1.00  6.66           N
ATOM   5881  CA  SER B 169      -2.522   9.730  29.550  1.00  6.73           C
ATOM   5883  CB  SER B 169      -1.937  10.743  28.561  1.00  6.97           C
ATOM   5886  OG  SER B 169      -0.786  10.274  27.893  1.00  7.01           O
ATOM   5888  C   SER B 169      -3.682  10.418  30.252  1.00  6.37           C
ATOM   5889  O   SER B 169      -4.809  10.457  29.735  1.00  7.00           O
ATOM   5890  N   PRO B 170      -3.423  11.031  31.401  1.00  6.55           N
ATOM   5891  CA  PRO B 170      -4.460  11.849  32.024  1.00  6.89           C
ATOM   5893  CB  PRO B 170      -3.857  12.207  33.376  1.00  7.40           C
ATOM   5896  CG  PRO B 170      -2.372  12.206  33.129  1.00  7.51           C
ATOM   5899  CD  PRO B 170      -2.132  11.112  32.117  1.00  7.19           C
ATOM   5902  C   PRO B 170      -4.681  13.102  31.183  1.00  6.85           C
ATOM   5903  O   PRO B 170      -3.735  13.676  30.622  1.00  7.40           O
ATOM   5904  N   VAL B 171      -5.937  13.524  31.132  1.00  7.09           N
ATOM   5906  CA  VAL B 171      -6.348  14.785  30.543  1.00  7.34           C
ATOM   5908  CB  VAL B 171      -7.465  14.557  29.506  1.00  7.54           C
ATOM   5910  CG1 VAL B 171      -7.909  15.888  28.901  1.00  8.25           C
ATOM   5914  CG2 VAL B 171      -7.031  13.593  28.430  1.00  7.81           C
ATOM   5918  C   VAL B 171      -6.840  15.593  31.737  1.00  7.34           C
ATOM   5919  O   VAL B 171      -7.955  15.357  32.214  1.00  8.22           O
ATOM   5920  N   PHE B 172      -5.982  16.449  32.278  1.00  7.59           N
ATOM   5922  CA  PHE B 172      -6.163  16.916  33.647  1.00  7.79           C
ATOM   5924  CB  PHE B 172      -5.221  16.170  34.623  1.00  8.27           C
ATOM   5927  CG  PHE B 172      -3.744  16.499  34.490  1.00  8.37           C
ATOM   5928  CD1 PHE B 172      -3.131  17.378  35.375  1.00  9.16           C
ATOM   5930  CE1 PHE B 172      -1.781  17.635  35.304  1.00  9.65           C
ATOM   5932  CZ  PHE B 172      -1.013  17.033  34.328  1.00  9.66           C
ATOM   5934  CE2 PHE B 172      -1.601  16.164  33.436  1.00  9.16           C
ATOM   5936  CD2 PHE B 172      -2.958  15.881  33.524  1.00  8.25           C
ATOM   5938  C   PHE B 172      -6.001  18.406  33.814  1.00  8.22           C
ATOM   5939  O   PHE B 172      -5.216  19.061  33.133  1.00  8.32           O
ATOM   5940  N   GLU B 173      -6.748  18.939  34.765  1.00  9.45           N
ATOM   5942  CA  GLU B 173      -6.530  20.289  35.261  1.00 10.25           C
ATOM   5944  CB  GLU B 173      -7.785  20.812  35.938  1.00 10.74           C
ATOM   5947  CG  GLU B 173      -8.990  20.794  35.029  1.00 11.64           C
ATOM   5950  CD  GLU B 173     -10.231  21.270  35.737  1.00 12.37           C
ATOM   5951  OE1 GLU B 173     -10.771  22.325  35.349  1.00 13.46           O
ATOM   5952  OE2 GLU B 173     -10.643  20.583  36.698  1.00 14.40           O
ATOM   5953  C   GLU B 173      -5.379  20.263  36.258  1.00 11.18           C
ATOM   5954  O   GLU B 173      -5.337  19.402  37.127  1.00 11.76           O
ATOM   5955  N   GLN B 174      -4.454  21.209  36.145  1.00 12.69           N
ATOM   5957  CA  GLN B 174      -3.289  21.244  37.026  1.00 14.03           C
ATOM   5959  CB  GLN B 174      -2.344  22.376  36.616  1.00 14.32           C
ATOM   5962  CG  GLN B 174      -1.682  22.176  35.261  1.00 14.57           C
ATOM   5965  CD  GLN B 174      -0.500  21.229  35.272  1.00 13.85           C
ATOM   5966  OE1 GLN B 174      -0.120  20.709  34.207  1.00 14.35           O
ATOM   5967  NE2 GLN B 174       0.089  20.999  36.440  1.00 13.83           N
ATOM   5970  C   GLN B 174      -3.670  21.420  38.499  1.00 14.98           C
ATOM   5971  O   GLN B 174      -3.055  20.828  39.382  1.00 15.06           O
ATOM   5972  N   SER B 175      -4.688  22.232  38.754  1.00 16.64           N
ATOM   5974  CA  SER B 175      -5.086  22.556  40.114  1.00 18.96           C
ATOM   5976  CB  SER B 175      -4.237  23.718  40.627  1.00 19.69           C
ATOM   5979  OG  SER B 175      -4.601  24.095  41.945  1.00 22.47           O
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ATOM   5981  C   SER B 175      -6.561  22.930  40.126  1.00 19.54           C
ATOM   5982  O   SER B 175      -6.933  24.006  39.666  1.00 20.78           O
ATOM   5983  N   SER B 176      -7.400  22.039  40.644  1.00 19.58           N
ATOM   5985  CA  SER B 176      -8.842  22.251  40.640  1.00 20.22           C
ATOM   5987  CB  SER B 176      -9.468  21.458  39.495  1.00 20.96           C
ATOM   5990  OG  SER B 176     -10.867  21.629  39.459  1.00 23.01           O
ATOM   5992  C   SER B 176      -9.475  21.805  41.947  1.00 20.01           C
ATOM   5993  O   SER B 176      -8.995  20.878  42.599  1.00 18.97           O
ATOM   5994  N   SER B 177     -10.560  22.486  42.311  1.00 20.67           N
ATOM   5996  CA  SER B 177     -11.457  22.038  43.368  1.00 21.82           C
ATOM   5998  CB  SER B 177     -11.738  23.164  44.369  1.00 22.03           C
ATOM   6001  OG  SER B 177     -12.180  24.343  43.719  1.00 24.65           O
ATOM   6003  C   SER B 177     -12.749  21.547  42.706  1.00 22.02           C
ATOM   6004  O   SER B 177     -13.563  22.340  42.230  1.00 23.51           O
ATOM   6005  N   ARG B 178     -12.881  20.226  42.622  1.00 21.68           N
ATOM   6007  CA  ARG B 178     -14.097  19.547  42.176  1.00 20.93           C
ATOM   6009  CB  ARG B 178     -13.937  18.996  40.745  1.00 20.43           C
ATOM   6012  CG  ARG B 178     -13.783  20.018  39.627  1.00 18.45           C
ATOM   6015  CD  ARG B 178     -13.677  19.382  38.238  1.00 16.30           C
ATOM   6018  NE  ARG B 178     -13.336  20.340  37.188  1.00 15.06           N
ATOM   6020  CZ  ARG B 178     -14.210  20.982  36.429  1.00 15.42           C
ATOM   6021  NH1 ARG B 178     -15.520  20.830  36.599  1.00 16.79           N
ATOM   6024  NH2 ARG B 178     -13.766  21.800  35.487  1.00 15.73           N
ATOM   6027  C   ARG B 178     -14.317  18.378  43.127  1.00 21.07           C
ATOM   6028  O   ARG B 178     -13.498  18.130  44.007  1.00 21.95           O
ATOM   6029  N   THR B 179     -15.409  17.643  42.952  1.00 20.41           N
ATOM   6031  CA  THR B 179     -15.601  16.424  43.723  1.00 20.45           C
ATOM   6033  CB  THR B 179     -16.934  15.754  43.349  1.00 21.16           C
ATOM   6035  OG1 THR B 179     -18.030  16.605  43.717  1.00 22.79           O
ATOM   6037  CG2 THR B 179     -17.156  14.483  44.160  1.00 22.10           C
ATOM   6041  C   THR B 179     -14.439  15.480  43.434  1.00 19.46           C
ATOM   6042  O   THR B 179     -14.150  15.185  42.267  1.00 19.41           O
ATOM   6043  N   ASN B 180     -13.759  15.050  44.493  1.00 18.57           N
ATOM   6045  CA  ASN B 180     -12.593  14.162  44.410  1.00 18.41           C
ATOM   6047  CB  ASN B 180     -12.948  12.851  43.684  1.00 18.60           C
ATOM   6050  CG  ASN B 180     -11.881  11.765  43.846  1.00 18.64           C
ATOM   6051  OD1 ASN B 180     -11.492  11.110  42.874  1.00 17.95           O
ATOM   6052  ND2 ASN B 180     -11.407  11.572  45.071  1.00 19.48           N
ATOM   6055  C   ASN B 180     -11.376  14.840  43.778  1.00 17.73           C
ATOM   6056  O   ASN B 180     -10.477  14.160  43.272  1.00 18.18           O
ATOM   6057  N   CYS B 181     -11.329  16.175  43.845  1.00 18.01           N
ATOM   6059  CA  CYS B 181     -10.170  16.955  43.412  1.00 17.47           C
ATOM   6061  CB  CYS B 181     -10.365  17.519  42.007  1.00 16.55           C
ATOM   6064  SG  CYS B 181     -10.449  16.203  40.788  1.00 14.03           S
ATOM   6065  C   CYS B 181      -9.864  18.092  44.372  1.00 18.41           C
ATOM   6066  O   CYS B 181     -10.756  18.845  44.780  1.00 19.23           O
ATOM   6067  N   ASN B 182      -8.595  18.188  44.734  1.00 19.10           N
ATOM   6069  CA  ASN B 182      -8.057  19.316  45.475  1.00 19.72           C
ATOM   6071  CB  ASN B 182      -8.215  19.085  46.989  1.00 20.52           C
ATOM   6074  CG  ASN B 182      -7.873  20.313  47.824  0.50 21.48           C
ATOM   6075  OD1 ASN B 182      -7.469  20.192  48.983  0.50 23.03           O
ATOM   6076  ND2 ASN B 182      -8.051  21.498  47.248  0.50 22.48           N
ATOM   6079  C   ASN B 182      -6.593  19.428  45.053  1.00 19.26           C
ATOM   6080  O   ASN B 182      -5.683  19.248  45.854  1.00 20.75           O
ATOM   6081  N   GLY B 183      -6.392  19.722  43.767  1.00 17.83           N
ATOM   6083  CA  GLY B 183      -5.102  19.586  43.106  1.00 16.31           C
ATOM   6086  C   GLY B 183      -5.308  19.065  41.691  1.00 15.03           C
ATOM   6087  O   GLY B 183      -6.328  19.348  41.063  1.00 15.07           O
ATOM   6088  N   PRO B 184      -4.353  18.300  41.168  1.00 13.71           N
ATOM   6089  CA  PRO B 184      -4.487  17.759  39.810  1.00 12.66           C
ATOM   6091  CB  PRO B 184      -3.241  16.889  39.651  1.00 13.12           C
ATOM   6094  CG  PRO B 184      -2.259  17.444  40.640  1.00 14.51           C
ATOM   6097  CD  PRO B 184      -3.077  17.925  41.800  1.00 14.52           C
ATOM   6100  C   PRO B 184      -5.769  16.941  39.671  1.00 11.46           C
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ATOM   6101  O   PRO B 184      -6.076  16.141  40.565  1.00 12.12           O
ATOM   6102  N   CYS B 185      -6.500  17.154  38.581  1.00 10.93           N
ATOM   6104  CA  CYS B 185      -7.851  16.641  38.453  1.00 10.37           C
ATOM   6106  CB  CYS B 185      -8.838  17.758  38.780  1.00 11.29           C
ATOM   6109  SG  CYS B 185     -10.536  17.205  38.967  1.00 13.15           S
ATOM   6110  C   CYS B 185      -8.095  16.139  37.046  1.00  9.18           C
ATOM   6111  O   CYS B 185      -8.272  16.933  36.118  1.00  9.91           O
ATOM   6112  N   SER B 186      -8.075  14.824  36.874  1.00  9.10           N
ATOM   6114  CA  SER B 186      -8.312  14.244  35.555  1.00  8.73           C
ATOM   6116  CB  SER B 186      -7.828  12.808  35.521  1.00  9.22           C
ATOM   6119  OG  SER B 186      -6.445  12.784  35.662  1.00 10.95           O
ATOM   6121  C   SER B 186      -9.792  14.276  35.205  1.00  8.85           C
ATOM   6122  O   SER B 186     -10.631  13.896  36.021  1.00  9.47           O
ATOM   6123  N   LEU B 187     -10.070  14.716  33.981  1.00  8.48           N
ATOM   6125  CA  LEU B 187     -11.417  14.803  33.438  1.00  8.48           C
ATOM   6127  CB  LEU B 187     -11.672  16.220  32.909  1.00  8.73           C
ATOM   6130  CG  LEU B 187     -11.486  17.345  33.923  1.00  9.71           C
ATOM   6132  CD1 LEU B 187     -11.768  18.686  33.266  1.00 10.15           C
ATOM   6136  CD2 LEU B 187     -12.372  17.141  35.147  1.00 11.50           C
ATOM   6140  C   LEU B 187     -11.677  13.791  32.329  1.00  8.23           C
ATOM   6141  O   LEU B 187     -12.828  13.573  31.958  1.00  8.64           O
ATOM   6142  N   ALA B 188     -10.612  13.181  31.805  1.00  8.18           N
ATOM   6144  CA  ALA B 188     -10.694  12.216  30.727  1.00  7.99           C
ATOM   6146  CB  ALA B 188     -10.950  12.910  29.397  1.00  7.96           C
ATOM   6150  C   ALA B 188      -9.385  11.425  30.699  1.00  7.51           C
ATOM   6151  O   ALA B 188      -8.414  11.769  31.366  1.00  7.88           O
ATOM   6152  N   VAL B 189      -9.389  10.372  29.896  1.00  7.53           N
ATOM   6154  CA  VAL B 189      -8.217   9.552  29.624  1.00  7.68           C
ATOM   6156  CB BVAL B 189      -8.268   8.135  30.229  0.35  8.28           C
ATOM   6157  CB AVAL B 189      -8.511   8.063  29.995  0.65  8.30           C
ATOM   6160  CG1BVAL B 189      -9.551   7.453  29.930  0.35  9.36           C
ATOM   6161  CG1AVAL B 189      -7.306   7.209  29.742  0.65  9.65           C
ATOM   6168  CG2BVAL B 189      -7.113   7.296  29.717  0.35 10.02           C
ATOM   6169  CG2AVAL B 189      -8.970   7.917  31.433  0.65  8.62           C
ATOM   6176  C   VAL B 189      -7.982   9.584  28.117  1.00  7.27           C
ATOM   6177  O   VAL B 189      -8.890   9.267  27.338  1.00  7.48           O
ATOM   6178  N   HIS B 190      -6.793   9.991  27.673  1.00  6.91           N
ATOM   6180  CA  HIS B 190      -6.546  10.097  26.248  1.00  6.84           C
ATOM   6182  CB  HIS B 190      -5.167  10.736  25.956  1.00  6.94           C
ATOM   6185  CG  HIS B 190      -4.917  10.787  24.504  1.00  6.68           C
ATOM   6186  ND1 HIS B 190      -5.791  11.423  23.659  1.00  7.50           N
ATOM   6188  CE1 HIS B 190      -5.449  11.150  22.417  1.00  7.27           C
ATOM   6190  NE2 HIS B 190      -4.369  10.394  22.428  1.00  7.70           N
ATOM   6192  CD2 HIS B 190      -4.006  10.160  23.732  1.00  7.53           C
ATOM   6194  C   HIS B 190      -6.656   8.714  25.580  1.00  6.62           C
ATOM   6195  O   HIS B 190      -6.168   7.735  26.122  1.00  7.01           O
ATOM   6196  N   THR B 191      -7.271   8.655  24.402  1.00  6.90           N
ATOM   6198  CA  THR B 191      -7.429   7.367  23.723  1.00  7.18           C
ATOM   6200  CB  THR B 191      -8.815   6.739  23.986  1.00  7.59           C
ATOM   6202  OG1 THR B 191      -9.845   7.700  23.751  1.00  9.16           O
ATOM   6204  CG2 THR B 191      -8.974   6.296  25.430  1.00  8.33           C
ATOM   6208  C   THR B 191      -7.162   7.340  22.221  1.00  7.39           C
ATOM   6209  O   THR B 191      -6.635   6.336  21.746  1.00  8.24           O
ATOM   6210  N   ASN B 192      -7.589   8.362  21.472  1.00  7.54           N
ATOM   6212  CA  ASN B 192      -7.637   8.270  20.016  1.00  8.61           C
ATOM   6214  CB  ASN B 192      -9.084   8.158  19.500  1.00 10.14           C
ATOM   6217  CG  ASN B 192      -9.884   7.097  20.205  1.00 13.02           C
ATOM   6218  OD1 ASN B 192      -9.925   5.949  19.768  1.00 17.47           O
ATOM   6219  ND2 ASN B 192     -10.571   7.484  21.269  1.00 13.77           N
ATOM   6222  C   ASN B 192      -7.053   9.497  19.349  1.00  7.81           C
ATOM   6223  O   ASN B 192      -7.187  10.604  19.845  1.00  7.54           O
ATOM   6224  N   GLY B 193      -6.466   9.272  18.178  1.00  7.89           N
ATOM   6226  CA  GLY B 193      -6.097  10.347  17.282  1.00  7.94           C
ATOM   6229  C   GLY B 193      -7.269  10.780  16.424  1.00  7.68           C
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ATOM   6230  O   GLY B 193      -8.434  10.495  16.712  1.00  8.59           O
ATOM   6231  N   VAL B 194      -6.934  11.448  15.329  1.00  7.94           N
ATOM   6233  CA  VAL B 194      -7.905  12.057  14.430  1.00  8.60           C
ATOM   6235  CB  VAL B 194      -7.210  13.166  13.608  1.00  9.21           C
ATOM   6237  CG1 VAL B 194      -8.096  13.671  12.465  1.00 10.61           C
ATOM   6241  CG2 VAL B 194      -6.800  14.308  14.504  1.00  9.07           C
ATOM   6245  C   VAL B 194      -8.484  10.982  13.518  1.00  9.30           C
ATOM   6246  O   VAL B 194      -7.749  10.269  12.840  1.00 10.19           O
ATOM   6247  N   TYR B 195      -9.806  10.861  13.489  1.00  9.47           N
ATOM   6249  CA  TYR B 195     -10.480   9.922  12.601  1.00 10.28           C
ATOM   6251  CB  TYR B 195     -10.327   8.471  13.092  1.00 11.06           C
ATOM   6254  CG  TYR B 195     -11.205   8.082  14.268  1.00 11.54           C
ATOM   6255  CD1 TYR B 195     -10.850   8.436  15.562  1.00 11.78           C
ATOM   6257  CE1 TYR B 195     -11.625   8.075  16.647  1.00 13.23           C
ATOM   6259  CZ  TYR B 195     -12.799   7.391  16.439  1.00 14.48           C
ATOM   6260  OH  TYR B 195     -13.573   7.033  17.518  1.00 17.11           O
ATOM   6262  CE2 TYR B 195     -13.188   7.044  15.160  1.00 15.20           C
ATOM   6264  CD2 TYR B 195     -12.393   7.385  14.082  1.00 13.19           C
ATOM   6266  C   TYR B 195     -11.953  10.279  12.489  1.00 10.34           C
ATOM   6267  O   TYR B 195     -12.463  11.132  13.209  1.00 10.36           O
ATOM   6268  N   GLY B 196     -12.644   9.603  11.582  1.00 11.32           N
ATOM   6270  CA  GLY B 196     -14.087   9.629  11.600  1.00 11.81           C
ATOM   6273  C   GLY B 196     -14.742  10.932  11.216  1.00 11.29           C
ATOM   6274  O   GLY B 196     -15.881  11.184  11.604  1.00 12.54           O
ATOM   6275  N   GLY B 197     -14.038  11.749  10.452  1.00 11.14           N
ATOM   6277  CA  GLY B 197     -14.556  13.043  10.072  1.00 11.40           C
ATOM   6280  C   GLY B 197     -14.354  14.124  11.118  1.00 10.86           C
ATOM   6281  O   GLY B 197     -14.712  15.268  10.864  1.00 11.89           O
ATOM   6282  N   SER B 198     -13.756  13.794  12.260  1.00 10.19           N
ATOM   6284  CA  SER B 198     -13.394  14.795  13.240  1.00  9.83           C
ATOM   6286  CB  SER B 198     -13.303  14.175  14.624  1.00  9.77           C
ATOM   6289  OG  SER B 198     -12.942  15.156  15.567  1.00  9.88           O
ATOM   6291  C   SER B 198     -12.066  15.428  12.891  1.00 10.01           C
ATOM   6292  O   SER B 198     -11.212  14.812  12.266  1.00 12.17           O
ATOM   6293  N   SER B 199     -11.898  16.664  13.339  1.00  9.97           N
ATOM   6295  CA  SER B 199     -10.645  17.378  13.227  1.00 10.64           C
ATOM   6297  CB  SER B 199     -10.911  18.863  12.962  1.00 11.88           C
ATOM   6300  OG  SER B 199     -11.618  19.054  11.760  1.00 15.50           O
ATOM   6302  C   SER B 199      -9.791  17.257  14.486  1.00  9.48           C
ATOM   6303  O   SER B 199      -8.720  17.848  14.532  1.00 10.66           O
ATOM   6304  N   TYR B 200     -10.239  16.495  15.480  1.00  8.05           N
ATOM   6306  CA  TYR B 200      -9.643  16.523  16.805  1.00  7.75           C
ATOM   6308  CB  TYR B 200     -10.654  17.084  17.810  1.00  7.69           C
ATOM   6311  CG  TYR B 200     -11.101  18.490  17.511  1.00  8.31           C
ATOM   6312  CD1 TYR B 200     -10.287  19.570  17.798  1.00  9.21           C
ATOM   6314  CE1 TYR B 200     -10.680  20.867  17.534  1.00 10.29           C
ATOM   6316  CZ  TYR B 200     -11.910  21.112  16.988  1.00 10.84           C
ATOM   6317  OH  TYR B 200     -12.299  22.414  16.730  1.00 13.51           O
ATOM   6319  CE2 TYR B 200     -12.751  20.065  16.697  1.00 11.29           C
ATOM   6321  CD2 TYR B 200     -12.345  18.748  16.960  1.00 10.10           C
ATOM   6323  C   TYR B 200      -9.217  15.133  17.266  1.00  7.26           C
ATOM   6324  O   TYR B 200      -9.662  14.114  16.746  1.00  8.16           O
ATOM   6325  N   ASN B 201      -8.348  15.125  18.274  1.00  7.06           N
ATOM   6327  CA  ASN B 201      -8.042  13.952  19.084  1.00  7.16           C
ATOM   6329  CB  ASN B 201      -6.680  14.153  19.748  1.00  7.15           C
ATOM   6332  CG  ASN B 201      -5.554  14.230  18.742  1.00  7.21           C
ATOM   6333  OD1 ASN B 201      -5.516  13.447  17.803  1.00  7.69           O
ATOM   6334  ND2 ASN B 201      -4.644  15.175  18.926  1.00  7.72           N
ATOM   6337  C   ASN B 201      -9.132  13.735  20.118  1.00  7.29           C
ATOM   6338  O   ASN B 201      -9.912  14.647  20.394  1.00  7.53           O
ATOM   6339  N   ARG B 202      -9.206  12.536  20.697  1.00  6.98           N
ATOM   6341  CA  ARG B 202     -10.279  12.211  21.629  1.00  7.41           C
ATOM   6343  CB  ARG B 202     -11.383  11.355  20.995  1.00  8.88           C
ATOM   6346  CG  ARG B 202     -11.693  11.653  19.568  1.00  9.57           C
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ATOM   6349  CD  ARG B 202     -12.972  11.011  19.099  1.00 10.98           C
ATOM   6352  NE  ARG B 202     -13.038  11.045  17.669  1.00 10.86           N
ATOM   6354  CZ  ARG B 202     -14.060  10.645  16.946  1.00 10.42           C
ATOM   6355  NH1 ARG B 202     -15.207  10.244  17.495  1.00 11.93           N
ATOM   6358  NH2 ARG B 202     -13.935  10.652  15.633  1.00 11.59           N
ATOM   6361  C   ARG B 202      -9.772  11.449  22.843  1.00  7.12           C
ATOM   6362  O   ARG B 202      -8.800  10.686  22.775  1.00  7.24           O
ATOM   6363  N   GLY B 203     -10.506  11.616  23.931  1.00  7.36           N
ATOM   6365  CA  GLY B 203     -10.273  10.888  25.156  1.00  7.64           C
ATOM   6368  C   GLY B 203     -11.594  10.478  25.782  1.00  7.46           C
ATOM   6369  O   GLY B 203     -12.600  11.167  25.693  1.00  8.95           O
ATOM   6370  N   THR B 204     -11.601   9.321  26.422  1.00  7.91           N
ATOM   6372  CA  THR B 204     -12.766   8.862  27.169  1.00  7.85           C
ATOM   6374  CB  THR B 204     -12.526   7.440  27.646  1.00  7.99           C
ATOM   6376  OG1 THR B 204     -12.283   6.626  26.490  1.00  9.14           O
ATOM   6378  CG2 THR B 204     -13.742   6.879  28.396  1.00  8.93           C
ATOM   6382  C   THR B 204     -13.049   9.778  28.339  1.00  7.48           C
ATOM   6383  O   THR B 204     -12.207   9.977  29.209  1.00  8.11           O
ATOM   6384  N   ARG B 205     -14.246  10.340  28.347  1.00  7.64           N
ATOM   6386  CA  ARG B 205     -14.673  11.241  29.393  1.00  8.00           C
ATOM   6388  CB  ARG B 205     -15.976  11.911  28.965  1.00  8.79           C
ATOM   6391  CG  ARG B 205     -16.504  12.958  29.902  1.00  8.59           C
ATOM   6394  CD  ARG B 205     -17.749  13.634  29.351  1.00  9.13           C
ATOM   6397  NE  ARG B 205     -18.197  14.685  30.247  1.00  9.66           N
ATOM   6399  CZ  ARG B 205     -19.108  15.593  29.932  1.00 11.19           C
ATOM   6400  NH1 ARG B 205     -19.463  16.494  30.836  1.00 12.79           N
ATOM   6403  NH2 ARG B 205     -19.631  15.622  28.720  1.00 12.68           N
ATOM   6406  C   ARG B 205     -14.893  10.499  30.697  1.00  8.17           C
ATOM   6407  O   ARG B 205     -15.442   9.398  30.704  1.00  8.50           O
ATOM   6408  N   ILE B 206     -14.511  11.107  31.803  1.00  7.90           N
ATOM   6410  CA  ILE B 206     -14.857  10.543  33.102  1.00  8.22           C
ATOM   6412  CB  ILE B 206     -13.888  10.984  34.205  1.00  8.53           C
ATOM   6414  CG1 ILE B 206     -12.479  10.503  33.832  1.00 10.37           C
ATOM   6417  CD1 ILE B 206     -11.395  10.782  34.838  1.00 11.44           C
ATOM   6421  CG2 ILE B 206     -14.335  10.417  35.576  1.00  8.96           C
ATOM   6425  C   ILE B 206     -16.304  10.954  33.378  1.00  8.18           C
ATOM   6426  O   ILE B 206     -16.577  12.055  33.837  1.00  9.63           O
ATOM   6427  N   THR B 207     -17.221  10.054  33.053  1.00  8.48           N
ATOM   6429  CA  THR B 207     -18.633  10.182  33.409  1.00  8.64           C
ATOM   6431  CB  THR B 207     -19.500   9.287  32.520  1.00  9.02           C
ATOM   6433  OG1 THR B 207     -19.159   7.926  32.815  1.00  9.39           O
ATOM   6435  CG2 THR B 207     -19.290   9.543  31.017  1.00  9.86           C
ATOM   6439  C   THR B 207     -18.829   9.725  34.857  1.00  8.82           C
ATOM   6440  O   THR B 207     -17.906   9.220  35.505  1.00  9.18           O
ATOM   6441  N   LYS B 208     -20.060   9.852  35.352  1.00  9.26           N
ATOM   6443  CA  LYS B 208     -20.369   9.298  36.665  1.00  9.84           C
ATOM   6445  CB  LYS B 208     -21.833   9.519  37.046  1.00 10.99           C
ATOM   6448  CG  LYS B 208     -22.087   9.129  38.528  1.00 14.71           C
ATOM   6451  CD  LYS B 208     -23.399   9.593  39.068  1.00 16.96           C
ATOM   6454  CE  LYS B 208     -23.489   9.258  40.552  1.00 19.57           C
ATOM   6457  NZ  LYS B 208     -23.241   7.822  40.859  1.00 20.47           N
ATOM   6461  C   LYS B 208     -20.034   7.814  36.745  1.00  9.27           C
ATOM   6462  O   LYS B 208     -19.537   7.336  37.761  1.00 10.16           O
ATOM   6463  N   GLU B 209     -20.331   7.079  35.694  1.00  9.18           N
ATOM   6465  CA  GLU B 209     -20.113   5.643  35.715  1.00  9.01           C
ATOM   6467  CB  GLU B 209     -20.903   4.935  34.624  1.00  9.53           C
ATOM   6470  CG  GLU B 209     -22.414   5.046  34.816  1.00 10.25           C
ATOM   6473  CD  GLU B 209     -22.978   6.405  34.428  1.00 10.76           C
ATOM   6474  OE1 GLU B 209     -23.862   6.914  35.155  1.00 12.33           O
ATOM   6475  OE2 GLU B 209     -22.549   6.961  33.386  1.00 11.21           O
ATOM   6476  C   GLU B 209     -18.624   5.295  35.653  1.00  8.78           C
ATOM   6477  O   GLU B 209     -18.183   4.353  36.318  1.00  9.54           O
ATOM   6478  N   VAL B 210     -17.843   6.052  34.878  1.00  8.31           N
ATOM   6480  CA  VAL B 210     -16.392   5.869  34.868  1.00  8.33           C
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ATOM   6482  CB  VAL B 210     -15.715   6.782  33.835  1.00  8.11           C
ATOM   6484  CG1 VAL B 210     -14.194   6.643  33.918  1.00  8.28           C
ATOM   6488  CG2 VAL B 210     -16.207   6.468  32.427  1.00  8.18           C
ATOM   6492  C   VAL B 210     -15.835   6.162  36.264  1.00  8.18           C
ATOM   6493  O   VAL B 210     -15.034   5.400  36.807  1.00  8.46           O
ATOM   6494  N   PHE B 211     -16.257   7.285  36.828  1.00  8.80           N
ATOM   6496  CA  PHE B 211     -15.865   7.717  38.169  1.00  8.95           C
ATOM   6498  CB  PHE B 211     -16.632   8.996  38.522  1.00  9.38           C
ATOM   6501  CG  PHE B 211     -16.350   9.534  39.891  1.00 10.33           C
ATOM   6502  CD1 PHE B 211     -17.036   9.054  40.992  1.00 12.42           C
ATOM   6504  CE1 PHE B 211     -16.794   9.562  42.250  1.00 14.20           C
ATOM   6506  CZ  PHE B 211     -15.867  10.570  42.422  1.00 14.22           C
ATOM   6508  CE2 PHE B 211     -15.184  11.071  41.328  1.00 12.56           C
ATOM   6510  CD2 PHE B 211     -15.427  10.548  40.077  1.00 10.94           C
ATOM   6512  C   PHE B 211     -16.144   6.610  39.183  1.00  9.01           C
ATOM   6513  O   PHE B 211     -15.284   6.254  39.997  1.00  9.62           O
ATOM   6514  N   ASP B 212     -17.341   6.057  39.145  1.00  9.24           N
ATOM   6516  CA  ASP B 212     -17.719   5.020  40.091  1.00  9.38           C
ATOM   6518  CB  ASP B 212     -19.220   4.742  40.000  1.00 10.22           C
ATOM   6521  CG  ASP B 212     -20.081   5.866  40.585  1.00 10.96           C
ATOM   6522  OD1 ASP B 212     -19.596   6.712  41.352  1.00 12.82           O
ATOM   6523  OD2 ASP B 212     -21.294   5.924  40.326  1.00 14.21           O
ATOM   6524  C   ASP B 212     -16.920   3.730  39.883  1.00  9.20           C
ATOM   6525  O   ASP B 212     -16.558   3.075  40.860  1.00  9.67           O
ATOM   6526  N   ASN B 213     -16.642   3.364  38.646  1.00  8.79           N
ATOM   6528  CA  ASN B 213     -15.823   2.182  38.386  1.00  8.75           C
ATOM   6530  CB BASN B 213     -15.892   1.765  36.925  0.35  8.75           C
ATOM   6531  CB AASN B 213     -15.742   1.816  36.880  0.65  8.93           C
ATOM   6536  CG BASN B 213     -17.240   1.173  36.556  0.35  9.22           C
ATOM   6537  CG AASN B 213     -16.833   0.837  36.379  0.65  9.63           C
ATOM   6538  OD1BASN B 213     -17.635   1.198  35.396  0.35 11.25           O
ATOM   6539  OD1AASN B 213     -17.182   0.862  35.191  0.65 11.89           O
ATOM   6540  ND2BASN B 213     -17.948   0.634  37.537  0.35  8.61           N
ATOM   6541  ND2AASN B 213     -17.315  -0.040  37.230  0.65  9.89           N
ATOM   6546  C   ASN B 213     -14.385   2.380  38.876  1.00  8.12           C
ATOM   6547  O   ASN B 213     -13.866   1.530  39.585  1.00  8.56           O
ATOM   6548  N   LEU B 214     -13.754   3.499  38.509  1.00  8.05           N
ATOM   6550  CA  LEU B 214     -12.388   3.756  38.975  1.00  8.09           C
ATOM   6552  CB  LEU B 214     -11.878   5.101  38.472  1.00  8.15           C
ATOM   6555  CG  LEU B 214     -11.645   5.232  36.974  1.00  8.61           C
ATOM   6557  CD1 LEU B 214     -11.247   6.665  36.638  1.00  9.83           C
ATOM   6561  CD2 LEU B 214     -10.596   4.247  36.475  1.00  9.91           C
ATOM   6565  C   LEU B 214     -12.321   3.712  40.498  1.00  8.01           C
ATOM   6566  O   LEU B 214     -11.378   3.153  41.070  1.00  8.50           O
ATOM   6567  N   THR B 215     -13.313   4.314  41.144  1.00  8.38           N
ATOM   6569  CA  THR B 215     -13.315   4.376  42.596  1.00  8.79           C
ATOM   6571  CB  THR B 215     -14.418   5.334  43.053  1.00  9.27           C
ATOM   6573  OG1 THR B 215     -14.177   6.633  42.485  1.00 10.10           O
ATOM   6575  CG2 THR B 215     -14.416   5.517  44.571  1.00 11.07           C
ATOM   6579  C   THR B 215     -13.481   2.979  43.209  1.00  8.80           C
ATOM   6580  O   THR B 215     -12.791   2.625  44.166  1.00  9.21           O
ATOM   6581  N   ASN B 216     -14.370   2.179  42.646  1.00  8.57           N
ATOM   6583  CA  ASN B 216     -14.557   0.818  43.115  1.00  8.67           C
ATOM   6585  CB  ASN B 216     -15.734   0.173  42.381  1.00  9.22           C
ATOM   6588  CG  ASN B 216     -15.982  -1.271  42.786  1.00  8.90           C
ATOM   6589  OD1 ASN B 216     -15.870  -1.642  43.963  1.00  9.87           O
ATOM   6590  ND2 ASN B 216     -16.303  -2.099  41.811  1.00 10.95           N
ATOM   6593  C   ASN B 216     -13.273  -0.002  42.920  1.00  8.34           C
ATOM   6594  O   ASN B 216     -12.861  -0.759  43.806  1.00  8.95           O
ATOM   6595  N   TRP B 217     -12.626   0.156  41.771  1.00  8.60           N
ATOM   6597  CA  TRP B 217     -11.442  -0.632  41.484  1.00  8.62           C
ATOM   6599  CB  TRP B 217     -11.051  -0.484  40.025  1.00  8.99           C
ATOM   6602  CG  TRP B 217     -12.086  -0.995  39.080  1.00  9.18           C
ATOM   6603  CD1 TRP B 217     -13.046  -1.934  39.324  1.00 10.15           C
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ATOM   6605  NE1 TRP B 217     -13.804  -2.145  38.197  1.00 11.24           N
ATOM   6607  CE2 TRP B 217     -13.350  -1.320  37.207  1.00 10.18           C
ATOM   6608  CD2 TRP B 217     -12.272  -0.584  37.733  1.00  9.00           C
ATOM   6609  CE3 TRP B 217     -11.640   0.346  36.907  1.00  9.73           C
ATOM   6611  CZ3 TRP B 217     -12.074   0.488  35.602  1.00 11.01           C
ATOM   6613  CH2 TRP B 217     -13.139  -0.262  35.117  1.00 11.64           C
ATOM   6615  CZ2 TRP B 217     -13.799  -1.163  35.897  1.00 11.72           C
ATOM   6617  C   TRP B 217     -10.303  -0.253  42.431  1.00  8.36           C
ATOM   6618  O   TRP B 217      -9.603  -1.117  42.953  1.00  8.91           O
ATOM   6619  N   LYS B 218     -10.123   1.033  42.695  1.00  8.79           N
ATOM   6621  CA  LYS B 218      -9.100   1.444  43.625  1.00  9.20           C
ATOM   6623  CB  LYS B 218      -8.827   2.934  43.515  1.00 11.18           C
ATOM   6626  CG  LYS B 218      -9.737   3.843  44.197  1.00 15.17           C
ATOM   6629  CD  LYS B 218      -9.326   5.287  43.946  1.00 18.59           C
ATOM   6632  CE  LYS B 218     -10.240   6.273  44.642  1.00 20.56           C
ATOM   6635  NZ  LYS B 218      -9.920   6.379  46.090  1.00 23.12           N
ATOM   6639  C   LYS B 218      -9.431   0.985  45.054  1.00  9.00           C
ATOM   6640  O   LYS B 218      -8.543   0.568  45.790  1.00 10.38           O
ATOM   6641  N   ASN B 219     -10.709   1.008  45.430  1.00  8.68           N
ATOM   6643  CA  ASN B 219     -11.124   0.530  46.752  1.00  8.69           C
ATOM   6645  CB  ASN B 219     -12.545   1.004  47.075  1.00  9.62           C
ATOM   6648  CG  ASN B 219     -12.589   2.441  47.549  1.00 10.94           C
ATOM   6649  OD1 ASN B 219     -11.678   2.906  48.223  1.00 14.01           O
ATOM   6650  ND2 ASN B 219     -13.697   3.138  47.267  1.00 11.68           N
ATOM   6653  C   ASN B 219     -11.040  -0.980  46.901  1.00  8.48           C
ATOM   6654  O   ASN B 219     -11.108  -1.494  48.016  1.00  9.89           O
ATOM   6655  N   SER B 220     -10.884  -1.688  45.792  1.00  8.99           N
ATOM   6657  CA  SER B 220     -10.799  -3.141  45.786  1.00  8.99           C
ATOM   6659  CB  SER B 220     -11.517  -3.693  44.555  1.00  9.46           C
ATOM   6662  OG  SER B 220     -12.907  -3.416  44.600  1.00  9.93           O
ATOM   6664  C   SER B 220      -9.357  -3.642  45.795  1.00  9.37           C
ATOM   6665  O   SER B 220      -9.124  -4.844  45.742  1.00 10.44           O
ATOM   6666  N   ALA B 221      -8.377  -2.741  45.851  1.00  9.78           N
ATOM   6668  CA  ALA B 221      -6.981  -3.155  45.805  1.00  9.87           C
ATOM   6670  CB  ALA B 221      -6.068  -1.948  45.804  1.00 10.44           C
ATOM   6674  C   ALA B 221      -6.632  -4.065  46.968  1.00 11.09           C
ATOM   6675  O   ALA B 221      -7.064  -3.848  48.094  1.00 12.58           O
ATOM   6676  N   GLN B 222      -5.824  -5.080  46.664  1.00 11.56           N
ATOM   6678  CA  GLN B 222      -5.345  -6.085  47.610  1.00 13.48           C
ATOM   6680  CB BGLN B 222      -5.070  -7.420  46.900  0.35 14.51           C
ATOM   6681  CB AGLN B 222      -5.003  -7.403  46.863  0.65 14.16           C
ATOM   6686  CG BGLN B 222      -3.617  -7.830  46.798  0.35 16.11           C
ATOM   6687  CG AGLN B 222      -6.230  -8.072  46.189  0.65 12.89           C
ATOM   6692  CD BGLN B 222      -3.455  -9.200  46.202  0.35 17.67           C
ATOM   6693  CD AGLN B 222      -5.908  -9.289  45.310  0.65 14.84           C
ATOM   6694  OE1BGLN B 222      -4.040 -10.165  46.695  0.35 19.06           O
ATOM   6695  OE1AGLN B 222      -4.806  -9.840  45.371  0.65 18.23           O
ATOM   6696  NE2BGLN B 222      -2.655  -9.300  45.148  0.35 18.44           N
ATOM   6697  NE2AGLN B 222      -6.880  -9.712  44.495  0.65 13.26           N
ATOM   6702  C   GLN B 222      -4.109  -5.562  48.352  1.00 14.27           C
ATOM   6703  O   GLN B 222      -3.636  -6.231  49.284  1.00 17.27           O
ATOM   6704  OXT GLN B 222      -3.579  -4.486  48.029  1.00 15.01           O
ATOM   6705 CA   CA  B 301      -0.643  21.256  17.293  1.00 10.41          CA
ATOM  13398  N   ASP F 401     -10.088   3.418  14.402  1.00 20.15           N
ATOM  13400  CA  ASP F 401     -10.419   4.298  15.551  1.00 19.20           C
ATOM  13402  CB  ASP F 401     -11.005   3.471  16.700  1.00 20.61           C
ATOM  13405  CG  ASP F 401     -12.475   3.140  16.497  1.00 22.97           C
ATOM  13406  OD1 ASP F 401     -13.045   2.395  17.327  1.00 26.18           O
ATOM  13407  OD2 ASP F 401     -13.144   3.572  15.537  1.00 25.29           O
ATOM  13408  C   ASP F 401      -9.196   5.076  16.021  1.00 16.65           C
ATOM  13409  O   ASP F 401      -9.239   5.713  17.069  1.00 16.48           O
ATOM  13412  N   ALA F 402      -8.115   5.032  15.242  1.00 14.63           N
ATOM  13414  CA  ALA F 402      -6.897   5.780  15.549  1.00 12.75           C
ATOM  13416  CB  ALA F 402      -7.112   7.245  15.277  1.00 12.61           C
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ATOM  13420  C   ALA F 402      -6.485   5.557  16.999  1.00 11.06           C
ATOM  13421  O   ALA F 402      -6.190   6.500  17.738  1.00 10.74           O
ATOM  13422  N   PHE F 403      -6.464   4.296  17.429  1.00 10.84           N
ATOM  13424  CA  PHE F 403      -6.076   4.009  18.798  1.00 10.34           C
ATOM  13426  CB  PHE F 403      -6.233   2.517  19.116  1.00 11.44           C
ATOM  13429  CG  PHE F 403      -7.671   2.025  19.183  1.00 12.38           C
ATOM  13430  CD1 PHE F 403      -8.562   2.511  20.119  1.00 14.77           C
ATOM  13432  CE1 PHE F 403      -9.880   2.048  20.187  1.00 17.09           C
ATOM  13434  CZ  PHE F 403     -10.309   1.064  19.322  1.00 18.48           C
ATOM  13436  CE2 PHE F 403      -9.424   0.544  18.386  1.00 18.39           C
ATOM  13438  CD2 PHE F 403      -8.109   1.018  18.324  1.00 16.19           C
ATOM  13440  C   PHE F 403      -4.626   4.428  19.018  1.00 10.00           C
ATOM  13441  O   PHE F 403      -3.748   4.110  18.209  1.00 12.25           O
ATOM  13442  N   GLU F 404      -4.372   5.130  20.116  1.00  8.64           N
ATOM  13444  CA  GLU F 404      -3.025   5.588  20.427  1.00  8.12           C
ATOM  13446  CB  GLU F 404      -2.992   7.120  20.524  1.00  7.95           C
ATOM  13449  CG  GLU F 404      -3.122   7.705  19.117  1.00  8.08           C
ATOM  13452  CD  GLU F 404      -3.043   9.212  19.009  1.00  7.71           C
ATOM  13453  OE1 GLU F 404      -3.129   9.917  20.027  1.00  8.61           O
ATOM  13454  OE2 GLU F 404      -2.901   9.672  17.856  1.00  8.80           O
ATOM  13455  C   GLU F 404      -2.442   4.854  21.637  1.00  8.22           C
ATOM  13456  O   GLU F 404      -2.865   3.708  21.892  1.00  8.82           O
ATOM  13457  OXT GLU F 404      -1.513   5.394  22.258  1.00  8.53           O
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